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(54) Title: SELECTING ANIMALS FOR PARENTALLY IMPRINTED TRAITS

(54) Titre: SELECTION D'ANIMAUX EN FONCTION DE TRAITS COMMUNIQUES PAR LEURS PARENTS

(57) Abstract

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. The invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7.

(57) Abrégé

L'invention concerne des procédés de sélection d'animaux reproducteurs ou destinés à l'abattoir sur la base des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles qui sont notamment liées à la masse musculaire et/ou aux dépôts de lard. L'invention se rapporte à un procédé pour sélectionner un porc possédant des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles, ledit procédé consistant à tester un échantillon provenant dudit porc pour vérifier la présence d'un locus quantitatif (QTL) présent dans la cartographie de chromosome 2 de Sus scrofa en position 2p1.7.

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The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. The invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7.

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L'invention concerne des procédés de sélection d'animaux reproducteurs ou destinés à l'abattoir sur la base des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles qui sont notamment liées à la masse musculaire et/ou aux dépôts de lard. L'invention se rapporte à un procédé pour sélectionner un porc possédant des propriétés génotypiques désirées ou des propriétés phénotypiques potentielles, ledit procédé consistant à tester un échantillon provenant dudit porc pour vérifier la présence d'un locus quantitatif (QTL) présent dans la cartographie de chromosome 2 de Sus scrofa en position 2p1.7.

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(72) Inventors; and (75) Inventors/Applicants (for US only): ANDERSS [SE/SE]; Bergagatan 30, S-752 39 Upps: GEORGES, Michel [BE/BE]; Rue Vieux Tige 2c Villers-aux-Tours (BE). SPINCEMAILLE, Geert Sint Denijsstraat 26, B-8550 Zwevegem (BE).	ila (S) I, B-31	E).	Without international search rep upon receipt of that report.	oort and to be republished
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Description

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Title: Selecting animals for parentally imprinted traits.

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been relied on so far.

The invention relates to methods to select breeding animals or animals destined for slaughter for having desired genotypic or potential phenotypic properties, in particular related to muscle mass and/or fat deposition. Breeding schemes for domestic animals have so far focused on farm performance traits and carcass quality. This has resulted in substantial improvements in traits like reproductive success, milk production, lean/fat ratio, prolificacy, growth rate and feed efficiency. Relatively simple performance test data have been the basis for these improvements, and selected traits were assumed to be influenced by a large number of genes, each of small effect (the infinitesimal gene model). There are now some important changes occurring in this area. First, the breeding goal of some breeding organisations has begun to include meat quality attributes in addition to the "traditional" production traits. Secondly, evidence is accumulating that current and new breeding goal traits may involve relatively large effects (known as major genes), as opposed to the infinitesimal model that has

Modern DNA-technologies provide the opportunity to exploit these major genes, and this approach is a very promising route for the improvement of meat quality, especially since direct meat quality assessment is not viable for potential breeding animals. Also for other traits such as lean/fat ratio, growth rate and feed efficiency, modern DNA technology can be very effective. Also these traits are not always easy to measure in the living animal.

The evidence for several of the major genes originally obtained using segregation analysis, i.e. without any DNA marker information. Afterwards molecular studies were performed to detect the location of these

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genes on the genetic map. In practice, and except for alleles of very large effect, DNA studies are required to dissect the genetic nature of most traits of economic importance. DNA markers can be used to localise genes or 5 alleles responsible for qualitative traits like coat . colour, and they can also be used to detect genes or alleles with substantial effects on quantitative traits like growth rate, IMF etc. In this case the approach is referred to as QTL (quantitative trait locus) mapping, wherein a QTL comprises at least a part of the nucleic acid genome of an animal where genetic information capable of influencing said quantitative trait (in said animal or in its offspring) is located. Information at DNA level can not only help to fix a specific major gene in a population, but also assist in the selection of a quantitative trait which is already selected for. Molecular information in addition to phenotypic data can increase the accuracy of selection and therefore the selection response.

Improving meat quality or carcass quality is not just about changing levels of traits like tenderness or marbling, but it is also about increasing uniformity. The existence of major genes provides excellent opportunities for improving meat quality because it allows large steps to be made in the desired direction. Secondly, it will help to reduce variation, since we can fix relevant genes in our products. Another aspect is that selecting for major genes allows differentiation for specific markets. Studies are underway in several species, particularly, pigs, sheep, deer and beef cattle.

In particular, intense selection for meat production has resulted in animals with extreme muscularity and leanness in several livestock species. In recent years it has become feasible to map and clone several of the genes causing these phenotypes, paving the way towards more efficient marker assisted selection, targeted drug development (performance enhancing products) and transgenesis. Mutations in the ryanodine receptor (Fuji

et al, 1991; MacLennan and Phillips, 1993) and myostatin (Grobet et al, 1997; Kambadur et al, 1997; McPherron and Lee, 1997) have been shown to cause muscular hypertrophies in pigs and cattle respectively, while genes with major effects on muscularity and/or fat deposition have for instance been mapped to pig chromosome 4 (Andersson et al, 1994) and sheep chromosome 18 (Cocket et al, 1996).

However, although there have been successes in identifying QTLs, the information is currently of limited use within commercial breeding programmes. Many workers in this field conclude that it is necessary to identify the particular genes underlying the QTL. This is a substantial task, as the QTL region is usually relatively large and may contain many genes. Identification of the relevant genes from the many that may be involved thus remains a significant hurdle in farm animals.

The invention provides a method for selecting a

domestic animal for having desired genotypic or potential
phenotypic properties comprising testing said animal for
the presence of a parentally imprinted qualitative or
quantitative trait locus (QTL). Herein, a domestic animal
is defined as an animal being selected or having been
derived from an animal having been selected for having
desired genotypic or potential phenotypic properties.

Domestic animals provide a rich resource of genetic and phenotypic variation, traditionally domestication involves selecting an animal or its offspring for having desired genotypic or potential phenotypic properties. This selection process has in the past century been facilitated by growing understanding and utilisation of the laws of Mendelian inheritance. One of the major problems in breeding programs of domestic animals is the negative genetic correlation between reproductive capacity and production traits. This is for example the case in cattle (a high milk production generally results

5 in slim cows and bulls) poultry, broiler lines have a low level of egg production and layers have generally very low muscle growth), pigs (very prolific sows are in general fat and have comparatively less meat) or sheep 10 (high prolific breeds have low carcass quality and vice versa). The invention now provides that knowledge of the parental imprinting character of various traits allows to select for example sire lines homozygous for a paternally 15 imprinted QTL for example linked with muscle production or growth; the selection for such traits can thus be less stringent in dam lines in favour of the reproductive quality. The phenomenon of genetic or parental imprinting 20 has never been utilised in selecting domestic animals, it. was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. The invention provides a breeding programme, wherein 25 knowledge of the parental imprinting character of a desired trait, as demonstrated herein, results in a breeding programme, for example in a BLUP programme, with a modified animal model. This increases the accuracy of 30 the breeding value estimation and speeds up selection compared to conventional breeding programmes. Until now, the effect of a parentally imprinted trait in the estimation of a conventional BLUP programme was 35 neglected; using and understanding the parental character of the desired trait, as provided by the invention, allows selecting on parental imprinting, even without DNA testing. For example, selecting genes characterised by 40 paternal imprinting is provided to help increase uniformity; a (terminal) parent homozygous for the "good or wanted" alleles will pass them to all offspring, regardless of the other parent's alleles, and the 45 offspring will all express the desired parent's alleles. This results in more uniform offspring. Alleles that are 35 interesting or favourable from the maternal side or often the ones that have opposite effects to alleles from the 50 paternal side. For example, in meat animals such as pigs alleles linked with meat quality traits such as inta-

\$5\$ muscular fat or muscle mass could be fixed in the dam lines while alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male lines.

In a preferred embodiment, the invention provides a method for selecting a domestic animal for having desired genotypic or potential phenotypic properties comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL). A nucleic acid sample can in general be obtained from various parts of the animal's body by methods known in the art. Traditional samples for the purpose of nucleic acid testing are blood samples or skin or mucosal surface samples, but samples from other tissues can be used as well, in particular sperm samples, oocyte or embryo samples can be used. In such a sample, the presence and/or sequence of a specific nucleic acid, be it DNA or RNA, can be determined with methods known in . 20 the art, such as hybridisation or nucleic acid amplification or sequencing techniques known in the art. The invention provides testing such a sample for the presence of nucleic acid wherein a QTL or allele associated therewith is associated with the phenomenon of parental imprinting, for example where it is determined whether a paternal or maternal allele of said QTL is capable of being predominantly expressed in said animal.

The purpose of breeding programs in livestock is to enhance the performances of animals by improving their genetic composition. In essence this improvement accrues by increasing the frequency of the most favourable alleles for the genes influencing the performance characteristics of interest. These genes are referred to as QTL. Until the beginning of the nineties, genetic improvement was achieved via the use of biometrical methods, but without molecular knowledge of the underlying QTL.

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Since the beginning of the nineties and due to recent developments in genomics, it is conceivable to identify the QTL underlying a trait of interest. The invention now provides identifying and using parentally imprinted QTLs which are useful for selecting animals by mapping quantitative trait loci. Again, the phenomenon of genetic or paternal imprinting has never been utilised in selecting domestic animals, it was never considered feasible to employ this elusive genetic characteristic in practical breeding programmes. For example Kovacs and Kloting (Biochem. Mol. Biol. Int. 44:399-405, 1998), where parental imprinting is not mentioned, and not suggested, found linkage of a trait in female rats, but not in males, suggesting a possible sex specificity associated with a chromosomal region, which of course excludes parental imprinting, a phenomenon wherein the imprinted trait of one parent is preferably but genderaspecifically expressed in his or her offspring.

The invention provides the initial localisation of a parentally imprinted QTL on the genome by linkage analysis with genetic markers, and the actual identification of the parentally imprinted gene(s) and causal mutations therein. Molecular knowledge of such a parentally imprinted QTL allows for more efficient breeding designs herewith provided. Applications of molecular knowledge of parentally imprinted QTLs in breeding programs include: marker assisted segregation analysis to identify the segregation of functionally distinct parentally imprinted QTL alleles in the populations of interest, marker assisted selection (MAS) performed within lines to enhance genetic response by increasing selection accuracy, selection intensity or by reducing the generation interval using the understanding of the phenomonon of parental imprinting, marker assisted introgression (MAI) to efficiently transfer favourable parentally imprinted QTL alleles from a donor to a recipient population, genetic engineering of the identified parentally QTL and genetic modification of the

breeding stock using transgenic technology, development

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of performance enhancing products using targeted drug development exploiting molecular knowledge of said QTL.

The inventors undertook two independent experiments to determine the practical use of parental imprinting of a QTL.

In a first experiment, performed in a previously described Piétrain x Large White intercross, the likelihood of the data were computed under a model of paternal (paternal allele only expressed) and maternal imprinting (maternal allele only expressed) and compared with the likelihood of the data under a model of a conventional "Mendelian" QTL. The results strikingly demonstrated that the QTL was indeed paternally expressed, the QTL allele (Piétrain or Large White) inherited from the F1 sow having no effect whatsoever on the carcass quality and quantity of the F2 offspring. It was seen that very significant lodscores were obtained when testing for the presence of a paternally expressed OTL, while there was no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. The same tendency was observed for all traits showing that the same imprinted gene is responsible for the effects observed on the different traits. Table 1 reports the maximum likelihood (ML) phenotypic means for the F2 offspring sorted by inherited paternal QTL allele.

In a second experiment performed in the Wild Boar X Large White intercross, QTL analyses of body composition, fatness, meat quality, and growth traits was carried out with the chromosome 2 map using a statistical model testing for the presence of an imprinting effect. Clear evidence for a paternally expressed QTL located at the very distal tip of 2p was obtained (Fig. 2; Tablel). The clear paternal expression of a QTL is illustrated by the least squares means which fall into two classes following the population origin of the paternally inherited allele (Table 1). For a given paternally imprinted QTL, implementation of marker assisted segregation analysis,

selection (MAS) and introgression (MAI), can be performed

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using genetic markers that are linked to the QTL, genetic markers that are in linkage disequilibrium with the QTL, or using the actual causal mutations within the QTL.

Understanding the parent-of-origin effect characterising a QTL allows for its optimal use in breeding programs. Indeed, marker assisted segregation analysis under a model of parental imprinting will yield better estimates of QTL allele effects. Moreover it allows for the application of specific breeding schemes to optimally exploit a QTL. In one embodiment of the invention, the most favourable QTL alleles would be fixed in breeding animal lines and for example used to generate commercial, crossbred males by marker assisted selection (MAS, within lines) and marker assisted introgression (MAI, between lines). In another embodiment, the worst QTL alleles would be fixed in the animal lines used to generate commercial crossbred females by MAS (within lines) and MAI (between lines).

In a preferred embodiment of the invention, said animal is a pig. Note for example that the invention provides the insight that today half of the offspring from commercially popular Piétrain_x Large White crossbred boars inherit an unfavourable Large White muscle mass QTL as provided by the invention causing considerable loss, and the invention now for example provides the possibility to select the better half of the population in that respect. However, it is also possible to select commercial sow lines enriched with the in the boars unfavourable alleles, allowing to equip the sows with

other alleles more desirable for for example reproductive

In a preferred embodiment of a method provided by the invention, said QTL is located at a position corresponding to a QTL located at chromosome 2 in the pig. For example, it is known form comparative mapping data between pig and human, including bidirectional chromosome painting, that SSC2p is homologous to HSAllpter-q13^{11,12}. HSAllpter-q13 is known to harbour a

purposes.

5 cluster of imprinted genes: IGF2, INS2, H19, MAH2, P57 KIP2, K,LQTL1, Tapa1,/CD81, Orctl2, Impt1 and Ip1. The cluster of imprinted genes located in HSAllpter-q13 is characterised by 8 maternally expressed genes H19, MASH2, P57KIP2, K,LQTL1, TAPA1/CD81, ORCTL2, IMPT1 and IP1, and 10 two paternally expressed genes: IGF2 and INS. However, Johanson et al (Genomics 25:682-690, 1995) and Reik et al (Trends in Genetics, 13:330-334, 1997) show that the 15 whereabouts of these loci in various animals are not clear. For example, the HSA11 and MMU7 loci do not correspond among each other, the MMU7 and the SSC2 loci do not correspond, whereas the HSA11 and SSC2 loci seem to correspond, and no guidance is given where one or more 20 of for example the above identified parentally expressed individual genes are localised on the three species' chromosomes. Other domestic animals, such as cattle, sheep, 25 poultry and fish, having similar regions in their genome harbouring such a cluster of imprinted genes or QTLs, the invention herewith provides use of these orthologous regions of other domestic animals in applying the 30 phenomenon of parental imprinting in breeding programmes. In pigs, said cluster is mapped at around position 2p1.7 of chromosome 2, however, a method as provided by the invention employing (fragments of) said maternally or 35 paternally expressed orthologous or homologous genes or QTLs are advantageously used in other animals as well for breeding and selecting purposes. For example, a method is provided wherein said QTL is related to the potential 40 muscle mass and/or fat deposition, preferably with limited effects on other traits such as meat quality and daily gain of said animal or wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) 45 allele. Reik et al (Trends in Genetics, 13:330-334, 1997) explain that this gene in humans is related to Beckwith-Wiedemann syndrome, an apparently parentally imprinted

disease syndrome most commonly seen with human foetuses,

where the gene has an important role in prenatal

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development. No relationship is shown or suggested with postnatal development relating to muscle development or fatness in (domestic) animals.

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In a preferred embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2pl.7. In particular, the invention relates to the use of genetic markers for the telomeric end of pig chromosome 2p in marker selection (MAS) of a parentally imprinted Quantitative Trait Locus (QTL) affecting carcass yield and quality in pigs. Furthermore, the invention relates to the use of genetic markers associated with the IGF2 locus in MAS in pigs, such as polymorphisms and microsatelites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. In a preferred embodiment, the invention provides a QTL located at the distal tip of Sus scrofa chromosomes 2 with effects on varies measurements of carcass quality and quantity, particularly muscle mass and fat deposition.

In a first experiment, a QTL mapping analysis was performed in a Wild Boar X Large White intercross counting 200 F_2 individuals. The F_2 animals were sacrificed at a live eight of at least 80 kg or at a maximum age of 190 days. Phenotypic data on birth weight, growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are provided by Andersson et al 4 and Andersson-Eklund et al 4

A QTL (without any significant effect on back-fat thickness) at an unspecified locus on the proximal end of chromosome 2 with moderate effect on muscle mass, and located about 30cM away from the parentally imprinted QTL reported here, was previously reported by the inventors; whereas the QTL as now provided has a very large effect, explaining at least 20-30% of variance, making the QTL of

the present invention commercially very attractive, which is even more so because the present QTL is parentally imprinted. The marker map of chromosome 2p was improved as part of this invention by adding microsatellite . markers in order to cover the entire chromosome arm. The 10 following microsatellite markers were used: Swc9, Sw2443, Sw2623, and Swr2516, all from the distal end of 2p7. QTL analyses of body composition, fatness, meat quality, and growth traits were carried out with the new chromosome 2 15 map. Clear evidence for a QTL located at the very distal 10 tip of 2p was obtained (Fig. 1; Table 1). The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic 20 variance in the F2 population. Large effects on the area of the longissumus dorsi muscle, on the weight of the 15 heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, 25 reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not 20 shown). The Large White allele at this QTL was associated 30 with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. In a second experiment, QTL mapping was performed in 25 35 a Piétrain X Large White intercross comprising 1125 F, offspring. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famous for their exceptional muscularity 40 and leanness 10 (Figure 2, while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring growth performance (5), muscularity (6), fat deposition (6), and meat quality (4), were recorded on 45 all F, offspring. In order to map QTL underlying the genetic differences between these breeds, the inventors 35 undertook a whole genome scan using microsatellite markers on an initial sample of 677 F, individuals. The 50 following microsatellite marker map was used to analyse

5		chromosome 2;:SW2443, SWC9 and SW2623, SWR2516-(0,20)- SWR783-(0,29)-SW240-(0,20)-SW776-(0,08)-S0010-(0,04)-
		SW1695-(0,36)-SWR308. Analysis of pig chromosome 2 using
•		a Maximum Likelihood multipoint algorithm, revealed
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10	5	highly significant lodscores (up to 20) for three of the
~		six phenotypes measuring muscularity (% lean cuts, % ham,
		% loin) and three of the six phenotypes measuring fat
		deposition (back-fat thickness (BFT), % backfat, % fat
15		cuts) at the distal end of the short arm of chromosome 2
	10	(Figure 1). Positive lodscores were obtained in the
		corresponding chromosome region for the remaining six
		muscularity and fatness phenotypes, however, not reaching
20		the experiment-wise significance threshold) (α =5%. There
		was no evidence for an effect of the corresponding QTL on
	15	growth performance (including birth weight) or recorded
		meat quality measurements (data not shown). To confirm
25		this finding, the remaining sample of 355 F_2 offspring was
		genotyped for the four most distal 2p markers and QTL
		analysis performed for the traits yielding the highest
	20	lodscores in the first analysis. Lodscores ranged from
30		2.1 to 7.7, clearly confirming the presence of a major
30		QTL in this region. Table 2 reports the corresponding ML
		estimates for the three genotypic means as well as the
		residual variance. Evidence based on marker assisted
	25	segregation analysis points towards residual segregation
35		at this locus within the Piétrain population.
		These experiments therefore clearly indicated
		the existence of a QTL with major effect on carcass
		quality and quantity on the telomeric end of pig
40	30 .	chromosome arm 2p; the likely existence of an allelic
		series at this QTL with at least three alleles: Wild-Boar
		< Large White < Piétrain, and possibly more given the
•		observed segregation within the Piétrain breed.
45		The effects of the identified QTL on muscle mass and
	35	fat deposition are truly major, being of the same
		magnitude of those reported for the CRC locus though
		taran da antara da a
50		apparently without the associated deleterious effects on
		meat quality. We estimate that both loci jointly explain

close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. The QTL had very large effects on lean meat content in ham and explained an astonishing 30% of the residual phenotypic variance in 5 the F_2 population. Large effects on the area of the longissumus dorsi muscle, on the weight of the heart, and on back-fat thickness (subcutaneous fat) were also noted. A moderate effect on one meat quality trait, reflectance value, was indicated. The QTL had no significant effect on abdominal fat, birth weight, growth, weight of liver, kidney, or spleen (data not shown). The Large White allele at this QTL, when compared to the Wild Boar allele, was associated with larger muscle mass and reduced back-fat thickness consistent with the difference between this breed and the Wild Boar population. The strong imprinting effect observed for all affected traits shows that a single causative locus is involved. The pleiotropic effects on skeletal muscle mass and the size of the heart appear adaptive from a physiological point 20 of view as a larger muscle mass requires a larger cardiac output.

In a further embodiment, the invention provides a method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 mapping at position 2p1.7., wherein said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele or a genonic area closely related thereto, such as polymorphisms and microsatelites and other characterising nucleic acid sequences shown herein, such as shown in figures 4 to 10. The important role of IGF2 for prenatal development is well-documented from knockout mice as well as from its causative role in the human Beckwith-Wiedemann syndrome. This invention demonstrates an important role for the IGF2-region also for postnatal development.

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To show the role of Igf2 the inventors performed the following three experiments:

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A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p.

A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IFG2 3 UTR using the BAC clone. A complex microsatellite was identified about 800bp downstream of the stop codon; a sequence comparison revealed that this microsatellite was identical to a previously described anonymous microsatellite, Swc96. This marker was used in the initial QTL mapping experiments and its location on the genetic map correspond with the most likely position of the QTL both in the Piétrain X Large White and in the Large White x Wild Boar pedigree.

Analysis of skeletal muscle and liver cDNA from 10week old foetuses heterozygous for a nt241 (G-A) transversion in the second exon of the porcine IGFII gene and SWC9, shows that the IGFII gene is imprinted in these tissues in the pig as well and only expressed from the paternal allele.

Based on a published porcine adult liver cDNA sequence16, the inventors designed primer pairs allowing to amplify the entire IgfII coding sequence with 222 bp of leader and 280 bp of trailor sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indication that the coding sequences are identical in both breeds and with the published sequence. However, a G⊠A transition was found in the leader sequence corresponding to exon 2 in man. Following conventional nomenclature, this polymorphism will be referred to as nt241(G-A). We developed a screening test for this single nucleotide polymorphism

15 5 9(SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, IgfII was shown to colocalize with the SWC9 microsatellite marker $(\theta=0\%)$, therefore virtually coinciding with the most likely position of the 10 QTL, and well within the 95% support interval for the QTL. Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3'UTR of the IgfII gene. 15 As previously mentioned, the knowledge of this 10 QTL provides a method for the selection of animals such as pigs with improved carcass merit. Different embodiments of the invention are envisaged, including: 20 marker assisted segregation analysis to identify the segregation of functionally distinct QTL alleles in the populations of interest; marker assisted selection (MAS) performed within lines to enhance genetic response by 25 increasing selection accuracy, selection intensity or by reducing the generation interval; marker assisted introgression (MAI) to efficiently transfer favourable QTL alleles from a donor to a recipient population; 30 thereby enhancing genetic response in the recipient population. Implementation of embodiments marker assisted segregation analysis, selection (MAS) and introgression (MAI), can be performed using genetic markers that are 35 linked to the QTL; genetic markers that are in linkage disequilibrium with the QTL, the actual causal mutations within the OTL. In a further embodiment, the invention provides a 40 method for selecting a pig for having desired genotypic or potential phenotypic properties comprising testing a sample from said pig for the presence of a quantitative trait locus (QTL) located at a Sus scrofa chromosome 2 45

mapping at position 2pl.7., wherein said QTL is paternally expressed, i.e. is expressed from the paternal allele. In man and mouse, Igf2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues. Analysis of skeletal muscle cDNA from

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pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in the pig as well. Understanding the parent-of-origin effect characterising the QTL as provided by the invention now allows for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss. Using a method as provide by the invention avoids this problem.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof comprising a parentally imprinted quantitative trait locus (QTL) or fragment thereof capable of being predominantly expressed by one parental allele. Having such a nucleic acid as provided by the invention available allows constructing transgenic animals wherein favourable genes are capable of being exclusively or predominantly expressed by one parental allele, thereby equipping the offspring of said animal homozygous for a desired trait with desired properties related to that parental allele that is expressed.

In a preferred embodiment, the invention provides an

isolated and/or recombinant nucleic acid or fragment derived thereof comprising a synthetic parentally imprinted quantitative trait locus (QTL) or functional fragment thereof derived from at least one chromosome. Synthetic herein describes a parentally expressed QTL wherein various elements are combined that originate from distinct locations from the genome of one or more animals. The invention provides recombinant nucleic acid wherein sequences related to parental imprinting of one QTL are combined with sequences relating to genes or favourable alleles of a second QTL. Such a gene construct is favourably used to obtain transgenic animals wherein the second QTL has been equipped with paternal imprinting, as opposed to the inheritance pattern in the native animal from which the second QTL is derived. Such a second QTL can for example be derived from the same

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chromosome where the parental imprinting region is located, but can also be dérived from a different chromosome from the same or even a different species. In the pig, such a second QTL can for example be related to an oestrogen receptor (ESR)-gene (Rothschild et al, PNAS, 93, 201-201, 1996) or a FAT-QTL (Andersson, Science, 263, 1771-1774, 1994) for example derived from an other pig chromosome, such as chromosome 4. A second or further QTL can also be derived from another (domestic) animal or a 10 human.

The invention furthermore provides an isolated and/or recombinant nucleic acid or functional fragment derived thereof at least partly corresponding to a OTL of a pig located at a Sus scrofa chromosome 2 mapping at position 2pl.7 wherein said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or wherein said QTL comprises at least a part of a Sus scrofa insulin-like growth factor-2 (IGF2) allele. preferably at least spanning a region between INS and H19, or preferably derived from a domestic pig, such as a 20 Pietrain, Meishan, Duroc, Landrace or Large White, or from a Wild Boar. For example, a genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong consistent signal on the terminal part of chromosome 2p. A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible presence of a corresponding porcine microsatellite was investigated by direct sequencing of the IGF2 3'UTR using the BAC clone. A complex microsatellite was identified about 800 bp downstream of the stop codon; a sequence comparison revealed that this microsatellite is identical to a previously described anonymous microsatellite, Swc9. PCR primers were designed and the microsatellite (IGF2ms) was

found to be highly polymorphic with three different

alleles among the two Wild Boar founders and another two

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among the eight Large White founders. IGF2ms was fully informative in the intercross as the breed of origin as well as the parent of origin could be determined with

confidence for each allele in each F_2 animal.

A linkage analysis using the intercross pedigree was carried out with IGF2ms and the microsatellites Sw2443, Sw2623, and Sw2516, all from the distal end of $2p^7$. IGF2 was firmly assigned to 2p by highly significant 1cd scores (e.g. Z=89.0, θ =0.003 against Sw2516). Multipoint

analyses, including previously typed chromosome 2 markers, revealed the following order of loci (sex-average map distances in Kosambi cM): Sw2443/Swr2516-0.3-IGF2-14.9-Sw2623-10.3-Sw256. No recombinant was observed between Sw2443 and Swr2516, and the suggested proximal

15 location of *IGF2* in relation to these loci is based on a single recombinant giving a lod score support of 0.8 for the reported order. The most distal marker in our previous QTL study, *Sw256*, is located about 25 cM from the distal end of the linkage group.

The invention furthermore provides use of a nucleic acid or functional fragment derived thereof according to the invention in a method according to the invention. In a preferred embodiment, use of a method according to invention is provided to select a breeding animal or

animal destined for slaughter, or embryos or semen derived from these animals for having desired genotypic or potential phenotypic properties. In particular, the invention provides such use wherein said properties are related to muscle mass and/or fat deposition. The QTL as

provided by the invention may be exploited or used to improve for example lean meat content or back-fat thickness by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another. Examples of marker

35 assisted selection using the QTL as provided by the invention are use of marker assisted segregation analysis

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with linked markers or with markers in disequilibrium to 5 identify functionally distinct QTL alleles. Furthermore, identification of a causative mutation in the QTL is now possible, again leading to identify functionally distinct QTL alleles. Such functionally distinct QTL alleles 10 located at the distal tip of chromosome 2p with large effects on skeletal muscle mass, the size of the heart, and on back-fat thickness are also provided by the 15 invention. The observation of a similar QTL effect in a Large White x Wild Boar as well as in a Piétrain x Large White intercross provides proof of the existence of a series of at least three distinct functional alleles. 20 Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series as provided 25 by the invention allows identifying causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but 20 rather subtle regulatory mutations. The effects on muscle 30 mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than Large White pigs that in turn have higher lean meat content than Wild Boars. The invention 35 furthermore provides use of the alleles as provided by 25 the invention for within line selection or for marker assisted introgression using linked markers, markers in disequilibrium or alleles comprising causative mutations. 40 The invention furthermore provides an animal 30 selected by using a method according to the invention. For example, a pig characterised in being homozygous for an allele in a QTL located at a:Sus scrofa chromosome 2 45 mapping at position 2pl.7 can now be selected and is thus provided by the invention. Since said QTL is related to the potential muscle mass and/or fat deposition of said pig and/or said QTL comprises at least a part of a Sus 50

scrofa insulin-like growth factor-2 (IGF2) allele, it is

possible to select promising pigs to be used for breeding 5 or to be slaughtered. In particular an animal according to the invention which is a male is provided. Such a male, or its sperm or an embryo derived thereof can. advantageously be used in breeding animals for creating 10 breeding lines or for finally breeding animals destined for slaughter. In a preferred embodiment of such use as provided by the invention, a male, or its sperm, deliberately selected for being homozygous for an allele 15 causing the extreme muscular hyperthrophy and leanness, is used to produce offspring heterozygous for such an allele. Due to said allele's paternal expression, said offspring will also show the favourable traits for 20 example related to muscle mass, even if the parent female 15 has a different genetic background. Moreover, it is now possible to positively select the female(s) for having different traits, for example related to fertility, 25 without having a negative effect on the muscle mass trait that is inherited from the allele from the selected male. For example, earlier such males could occasionally be seen with Piétrain pigs but genetically it was not 30 understood how to most profitably use these traits in breeding programmes. Furthermore, the invention provides a transgenic animal, sperm and an embryo derived thereof, comprising a 35 synthetic parentally imprinted QTL or functional fragment thereof as provided by the invention, i.e. it is provided by the invention to introduce a favourable recombinant allele; for example introduce the oestrogen receptor 40 locus related to increased litter size of an animal homozygously in a parentally imprinted region of a grandparent animal (for example the father of a hybrid sow if the region was paternally imprinted and the 45 grandparent was a boar); to introduce a favourable fatrelated allele or muscle mass-related recombinant allele in a paternally imprinted region, and so on. Recombinant alleles that are interesting or favourable from the 50 maternal side or often the ones that have opposite effects to alleles from the paternal side. For example,

in meat animals such as pigs recombinant alleles linked with meat quality traits such as intra-muscular fat or muscle mass could be fixed in the dam lines while recombinant alleles linked with reduced back fat could be fixed in the sire lines. Other desirable combinations are 10 for example fertility and/or milk yield in the female line with growth rates and/or muscle mass in the male The invention is further explained in the detailed 15 description without limiting the invention. Detailed description. 20 Example 1: Wild Boar x Large White intercrosses 15 Methods 25 Isolation of an IGF2 BAC clone and fluorescent in situ hybridization (FISH). IGF2 primers (F:5'-GGCAAGTTCTTCCGCTAATGA-3' and R:5'-GCACCGCAGAATTACGACAA-3') for PCR amplification of a part of the last exon and 30 3'UTR were designed on the basis of a porcine IGF2 cDNA sequence (GenBank X56094). The primers were used to screen a porcine BAC library and the clone 253G10 was 35 25 isolated. Crude BAC DNA was prepared as $described^{24}$. The BAC DNA was linearized with EcoRV and purified with QIAEXII (QIAGEN GmbH, Germany). The clone was labeled with biotin-14-dATP using the GIBCO-BRL Bionick labeling 40 system (BRL18246-015). Porcine metaphase chromosomes were obtained from pokeweed (Seromed) stimulated lymphocytes using standard techniques. The slides were aged for two 45 days at room temperature and then kept at -20°C until use. FISH analysis was carried out as previously ${\tt described}^{25}.$ The final concentration of the probe in the hybridization mix was 10 $ng/\mu l$. Repetitive sequences were 50

suppressed with standard concentrations of porcine

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			22	PC1/EP99/1020
5		genomic DNA. A	fter post-hybridizatio	n washing, the
		biotinylated p	robe was detected with	two layers of
		avidin-FITC (V	ector A-2011). The chr	omosomes were
		counterstained	with 0.3 mg/ml DAPI (4,6-Diamino-2-
10	5	phenylindole;	Sigma D9542), which pr	oduced a G-banding
		like pattern.	No posthybridization b	anding was needed,
		since chromoso	me 2 is easily recogni	zed without banding.
45		A total of 20	metaphase spreads were	examined under an
15		Olympus BX-60	fluorescence microscop	e connected to an
	10	IMAC-CCD S30 v	ideo camera and equipp	ed with an ISIS 1.65
		(Metasystems)	software.	
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		Sequence, micr	osatellite, and linkag	e analysis.
	15	About two µg o	f linearized and purif	ied BAC DNA was used
25		for direct seq	uencing with 20 pmoles	of primers and
			tor chemistry (Perkin	
			done from the 3' end	
		towards the 3'	end of the UTR until a	a microsatellite was
30	20	a.	imer set (F:5'-GTTTCTC	
			orescein- CTACAAGCTGG	
	,		ne amplification of the	
			250 bp long and locate	
35	ř.		from the stop codon. The	
	25		using fluorescently lab	
			carried out using an A	
40	•		enotyper softwares (Per	
			nultipoint linkage ana	lysis were done with
		the Cri-Map so	ftware ²⁶ .	
	30			
45		Animals and phe	enotypic data.	

The intercross pedigree comprised two European Wild Boar males and eight Large White females, 4 $F_{\rm 1}$ males and 22 $F_{\rm 1}$ 35 females, and 200 $F_2\ progeny^{\textstyle 1}.$ The F_2 animals were sacrificed at a live weight of at least 80 kg or at a

maximum age of 190 days. Phenotypic data on birth weight, 5 growth, fat deposition, body composition, weight of internal organs, and meat quality were collected; a detailed description of the phenotypic traits are 10 5 provided by Andersson et al. 1 and Andersson-Eklund et a1.4 Statistical analysis. 15 Interval mapping for the presence of QTL were carried out with a least squares method developed for the analysis of crosses between outbred lines 27 . The method is based on 20 the assumption that the two divergent lines are fixed for alternative QTL alleles. There are four possible genotypes in the F2 generation as regards the 25 grandparental origin of the alleles at each locus. This makes it possible to fit three effects: additive, dominance, and imprinting². The latter is estimated as the difference between the two types of heterozygotes, 30 the one receiving the Wild Boar allele through an F_1 sire and the one receiving it from an F1 dam. An F-ratio was calculated using this model (with 3 d.f.) versus a reduced model without a QTL effect for each cM of 35 chromosome 2. The most likely position of a QTL was obtained as the location giving the highest F-ratio. Genome-wise significance thresholds were obtained 40 empirically by a permutation test 28 as described 2 . The QTL model including an imprinting effect was compared with a model without imprinting (with 1 d.f.) to test whether the imprinting effect was significant. 45 The statistical models also included the fixed effects and covariates that were relevant for the

effects and covariates that were relevant for the respective traits; see Andersson-Eklund et al.⁴ for a more detailed description of the statistical models used.

35 Family was included to account for background genetic

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effects and maternal effects. Carcass weight was included 5 as a covariate to discern QTL effects on correlated traits, which means that all results concerning body composition were compared at equal weights. Least-squares 10 5 means for each genotype class at the IGF2 locus were estimated with a single point analysis using Procedure GLM of SAS²⁹; the model included the same fixed effects and covariates as used in the interval mapping analyses. 15 The QTL shows a clear parent of origin-specific expression and the map position coincides with that of the insulin-like growth factor II gene (IGF2), indicating IGF2 as the causative gene. A highly significant 20 segregation distortion (excess of Wild Boar-derived alleles) was also observed at this locus. The results 15 demonstrate an important effect of the IGF2 region on postnatal development and it is possible that the 25 presence of a paternally expressed IGF2-linked QTL in humans and in rodent model organisms has so far been overlooked due to experimental design or statistical 30 20 treatment of data. The study has also important implications for quantitative genetics theory and practical pig breeding. IGF2 was identified as a positional candidate gene 35 for this QTL due to the observed similarity between pig 25 chromosome 2p and human chromosome 11p. A genomic IGF2 clone was isolated by screening a porcine BAC library. FISH analysis with this BAC clone gave a strong 40 consistent signal on the terminal part of chromosome 2p (Fig. 1). A polymorphic microsatellite is located in the 3'UTR of IGF2 in mice (GenBank U71085), humans (GenBank S62623), and horse (GenBank AF020598). The possible 45 presence of a corresponding porcine microsatellite was investigated by direct sequencing of the 1GF2 3'UTR using the BAC clone. A complex microsatellite was identified 50 about 800 bp downstream of the stop codon; a sequence

comparison revealed that this microsatellite is identical

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5		to a previously described anonymous microsatellite,
		Swc96. PCR primers were designed and the microsatellite
		(IGF2ms) was found to be highly polymorphic with three
		different alleles among the two Wild Boar founders and
10	5	another two among the eight Large White founders. IGF2ms
		was fully informative in the intercross as the breed of
		origin as well as the parent of origin could be
		determined with confidence for each allele in each F_2
15		animal.
	10	A linkage analysis using the intercross pedigree was
\		carried out with $IGF2ms$ and the microsatellites $Sw2443$,
20		$Sw2623$, and $Swr2516$, all from the distal end of $2p^7$. $IGF2$
		was firmly assigned to 2p by highly significant lod
		scores (e.g. Z=89.0, θ =0.003 against $Swr2516$). Multipoint
	15	analyses, including previously typed chromosome 2
25		markers ⁸ , revealed the following order of loci (sex-
	•	average map distances in Kosambi cM): Sw2443/Swr2516-0.3-
•		IGF2-14.9-Sw2623-10.3-Sw256. No recombinant was observed
20		between Sw2443 and Swr2516, and the suggested proximal
30	20	location of IGF2 in relation to these loci is based on a
		single recombinant giving a lod score support of 0.8 for
		the reported order. The most distal marker in our
35	•	previous QTL study, Sw256, is located about 25 cM from
		the distal end of the linkage group.
	25	QTL analyses of body composition, fatness, meat
		quality, and growth traits were carried out with the new
40		chromosome 2 map using a statistical model testing for
		the possible presence of an imprinting effect as expected
		for IGF2. Clear evidence for a paternally expressed QTL
	30	located at the very distal tip of 2p was obtained (Fig.
45		2; Table 1). The QTL had very large effects on lean meat
		content in ham and explained an astonishing 30% of the
	•	residual phenotypic variance in the F ₂ population. Large
		Large

effects on the area of the longissumus dorsi muscle, on

the weight of the heart, and on back-fat thickness

5		(subcutaneous fat) were also noted. A moderate effect on
		one meat quality trait, reflectance value, was indicated
		The QTL had no significant effect on abdominal fat, birth
		weight, growth, weight of liver, kidney, or spleen (data
10	5	not shown). The Large White allele at this QTL was
		associated with larger muscle mass and reduced back-fat
•		thickness consistent with the difference between this
		breed and the Wild Boar population. The strong imprinting
15		effect observed for all affected traits strongly suggests
	10	a single causative locus. The pleiotropic effects on
		skeletal muscle mass and the size of the heart appear
20		adaptive from a physiological point of view as a larger
20		muscle mass requires a larger cardiac cutput. The clear
		paternal expression of this QTL is illustrated by the
	15	least squares means which fall into two classes following
25		the population origin of the paternally inherited allele
20		(Table 1). It is worth noticing though that there was a
		non-significant trend towards less extreme values for the
		two heterozygous classes, in particular for the estimated
30	20	effect on the area of longissimus dorsi. This may be due
		to chance, but could have a biological explanation, e.g.
		that there is some expression of the maternally inherited
		allele or that there is a linked, non-imprinted QTL with
35	-	minor effects on the traits in question. \sim
	25	The $IGF2$ -linked QTL and the $FAT1$ QTL on chromosome 4
•		1 , 9 are by far the two loci with the largest effect on
		body composition and fatness segregating in this Wild
40		Boar intercross. The IGF2 QTL controls primarily muscle
		mass whereas FAT1 has major effects on fat deposition
	30	including abdominal fat, a trait that was not affected by
45		the IGF2 QTL (Fig. 2). No significant interaction between
40		the two loci was indicated and they control a very large
		proportion of the residual phenotypic variance in the F_2
	•	generation. A model including both QTLs explains 33.1% of
50	35	the variance for percentage lean meat in ham, 31.3% for
		. 5 51.56 101

the percentage of lean meat plus bone in back, and 26.2%

for average back fat depth (compare with a model including only chromosome 2 effects, Table 1). The two QTLs must have played a major role in the response during selection for lean growth and muscle mass in the Large White domestic pig.

A highly significant segregation distortion was observed in the IGF2 region (excess of Wild Boar-derived alleles) as shown in Table 1 (χ 2=11.7, d.f.=2; P=0.003). The frequency of Wild Boar-derived IGF2 alleles was 59% in contrast to the expected 50% and there was twice as many "Wild Boar" as "Large White" homozygotes. This deviation was observed with all three loci at the distal tip and is thus not due to typing errors. The effect was also observed with other loci but the degree of distortion decreased as a function of the distance to the distal tip of the chromosome. Blood samples for DNA preparation were collected at 12 weeks of age and we are convinced that the deviation from expected Mendelian ratios was present at birth as the number of animals lost prior to blood sampling was not sufficient to cause a deviation of this magnitude. No other of the more than 250 loci analyzed in this pedigree show such a marked segregation distortion (L. Andersson, unpublished). The segregation distortion did not show an imprinting effect, as the frequencies of the two reciprocal types of heterozygotes were identical (Table 1). This does not exclude the possibility that the QTL effects and the segregation distortion are controlled by the same locus. The segregation distortion maybe due to meiotic drive 30 favoring the paternally expressed allele during gametogenesis, as the F1 parents were all sired by Wild Boar males. Another possibility is that the segregation distortion may be due to codominant expression of the maternal and paternal allele in some tissues and/or

during a critical period of embryo development. Biallelic

IGF2 expression has been reported to occur to some extent

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during human development 10 , 11 and interestingly a strong influence of the parental species background on IGF2 expression was recently found in a cross between Mus musculus and Mus spretus12. It is also interesting that a 10 VNTR polymorphism at the insulin gene, which is very closely linked to IGF2, is associated with size at birth in humans 13 . It is possible that the IGF2-linked QTL in pigs has a minor effect on birth weight but in our data

> indication of an imprinting effect. . 10

This study is an advance in the general knowledge concerning the biological importance of the IGF2 locus. The important role of IGF2 for prenatal development is well-documented from knock-out mice 14 as well as from its causative role in the human Beckwith-Wiedemann $syndrome^{15}$. This study demonstrates an important role for the IGF2-region also for postnatal development. It should be stressed that our intercross between outbred populations is particularly powerful to detect QTL with a parent of origin-specific effect on a multifactorial trait. This is because multiple alleles (or haplotypes) are segregating and we could deduce whether a heterozygous F2 anima: received the Wild Boar allele from the F_1 male or female. It is quite possible that the segregation of a paternally expressed IGF2-linked QTL affecting a trait like obesity has been overlooked in human studies or in intercrosses between inbred rodent populations because of experimental design or statistical

it was far from significant (Fig. 2) and there was no

detected in an intercross between two inbred lines as only two alleles are segregating at each locus. Our result has therefore significant bearings on the future analysis of the association between genetic polymorphism in the insulin-IGF2 region and Type I diabetes 16,

treatment of data. An imprinting effect cannot be

35 obesity 17, and variation in birth weight 13 in humans, as

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5 well as for the genetic dissection of complex traits using inbred rodent models. A major impetus for generating an intercross between the domestic pig and its wild ancestor was to explore the possibilities to map and 10 identify major loci that have responded to selection. We have now showed that two single QTLs on chromosome 2 (this study) and 4^{1} , 2 explain as much as one third of the phenotypic variance for lean meat content in the F_2 15 generation. This is a gross deviation from the underlying assumption in the classical infinitesimal model in quantitative genetics theory namely that quantitative traits are controlled by an infinite number of loci each 20 with an infinitesimal effect. If a large proportion of the genetic difference between two divergent populations (e.g. Wild Boar and Large White) is controlled by a few loci, one would assume that selection would quickly fix 25 QTL alleles with large effects leading to a selection plateau. However, this is not the experience in animal breeding programs or selection experiments where good 30 persistent long-term selection responses are generally obtained, provided that the effective population size is reasonably large 18. A possible explanation for this paradox is that QTL alleles controlling a large 35 proportion of genetic differences between two populations may be due to several consecutive mutations; this may be mutations in the same gene or at several closely linked genes affecting the same trait. It has been argued that 40 new mutations contribute substantially to long-term selection responses 19, but the genomic distribution of 3.0 such mutations are unknown. 45 The search for a single causative mutation is the paradigm as regards the analysis of genetic defects in

The search for a single causative mutation is the paradigm as regards the analysis of genetic defects in mice and monogenic disorders in humans. We propose that this may not be the case for loci that have been under selection for a large number of generations in domestic animals, crops, or natural populations. This hypothesis

predicts the presence of multiple alleles at major OTL. It gains some support from our recent characterization of porcine coat color variation. We have found that both the alleles for dominant white color and for black-spotting 10 differ from the corresponding wild-type alleles by at least two consecutive mutations with phenotypic effects at the KIT and MCIR loci, respectively 20, 21. In this context it is highly interesting that in the accompanying 15 example we have identified a third allele at the IGF2linked QTL. The effects on muscle mass of the three alleles rank in the same order as the breeds in which they are found i.e. Piétrain pigs are more muscular than 20 Large White pigs that in turn have higher lean meat content than Wild Boars. There are good reasons to decide that IGF2 is the 15 causative gene for the now reported QTL. Firstly, there 25 is a perfect agreement in map localization (Fig. 2). Secondly, it has been shown that IGF2 is paternally expressed in mice, humans, and now in pigs, like the QTL. 30 20 There are several other imprinted genes in the near vicinity of IGF2 in mice and humans (Mash2, INS2, H19, KVLQT1, TAPA1/CD81, and CDKN1C/p57K1P2) out only IGF2 is paternally expressed in adult tissues²². We believe that 35 this locus provides a unique opportunity for molecular characterization of a QTL. The clear paternal expression can be used to exclude genes that do not show this mode of inheritance. Moreover, the presence of an allelic 40 series should facilitate the difficult distinction between causative mutations and linked neutral polymorphism. We have already shown that there is no difference in coding sequence between IGF2 alleles from 45 Piétrain and Large White pigs suggesting that the causative mutations occur in regulatory sequences. An

obvious step is to sequence the entire IGF2 gene and its

multiple promoters from the three populations. The recent

report that a VNTR polymorphism in the promoter region of

the insulin (INS) gene affects IGF2 expression²³ suggests that the causative mutations may be at a considerable distance from the IGF2 coding sequence.

The results have several important implications for the pig breeding industry. They show that genetic imprinting is not an esoteric academic question but need to be considered in practical breeding programs. The detection of three different alleles in Wild Boar, Large

10 White, and Piétrain populations indicates that further alleles at the *IGF2*-linked QTL segregate within commercial populations. The paternal expression of the QTL facilitates its detection using large paternal halfsib families as the female contribution can be ignored.

15 The QTL is exploited to improve lean meat content by marker assisted selection within populations or by marker assisted introgression of favorable alleles from one population to another.

Example 2: Piétrain x Large White intercrosses

Methods

Pedigree material: The pedigree material utilized to map

QTL was selected from a previously described Piétrain x

Large White F2 pedigree comprising > 1,800 individuals^{6,7}.

To assemble this F2 material, 27 Piétrain boars were
mated to 20 Large White sows to generate an F1 generation
comprising 456 individuals. 31 F1 boars were mated to

unrelated 82 F1 sows from 1984 to 1989, yielding a total
of 1862 F2 offspring. F1 boars were mated on average to 7
females, and F1 sows to an average of 2,7 males. Average
offspring per boar were 60 and per sow 23.

- Phenotypic information: (i) Data collection: A total of
 21 distinct phenotypes were recorded in the F2
 generation^{6,7}. These included:
 - five growth traits: birth weight (g), weaning weight (Kg), grower weight (Kg), finisher weight (Kg) and
- 20 average daily gain (ADG; Kg/day; grower to finsher period);
 - two body proportion measurements: carcass length (cm); and a conformation score (0 to 10 scale; ref.6);
 - ten measurements of carcass composition obtained by
- dissection of the chilled carcasses 24 hours after
 slaughter. These include measurements of muscularity: %
 ham (weight hams/carcass weight), % loin (weight
 loin/carcass weight), % shoulder (weight
 shoulder/carcass weight), % lean cuts (% ham + %loin + %
- shoulder); and measurements of fatness: average back-fat thickness (BFT; cm), % backfat (weight backfat/carcass weight), % belly (weight belly/carcass weight), % leaf fat (weight leaf fat/carcass weight), % jowl (weight jowl/carcass weight), and "% fat cuts" (% backfat + %
- 35 belly + % leaft fat + % jowl).
 - four meat quality measurements: pH $_{ t LD1}$ (${\it Longissimus dorsi}$ 1

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hour after slaughter), pH _{LD24} (*Longissimus dorsi* 24 hours after slaughter), pH _{G1} (*Gracilis* 1 hour after slaughter) and pH _{G24} (*Gracilis* 24 hours after slaughter). (ii) Data processing: Individual phenotypes were preadjusted for fixed effects (sire, dam, CRC genotype, sex, year-season, parity) and covariates (litter size, birth weight, weaning weight, grower weight, finisher weight) that proved to significantly affect the corresponding trait. Variables included in the model were selected by stepwise regression.

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Marker genotyping: Primer pairs utilized for PCR amplification of microsatellite markers are as described¹⁹. Marker genotyping was performed as previously described²⁰. Genotypes at the CRC and MyoD loci were determined using conventional methods as described^{1,12}. The LAR test for the Igf2 SNP was developed according to Baron et al.²¹ using a primer pair for PCR amplification (5'-CCCCTGAACTTGAGGACGAGCC-3';5'-ATCGCTGTGGGCTGGGTGGCCCCC-3') and a set of three primers for the LAR step (5'-FAM-CGCCCCAGCTGCCCCCCAA-3';5'-HEX-CGCCCCAGCTGCCCCCCAA-3';5'-

CCTGAGCTGCAGCAGGCCAG-3').

Map construction: Marker maps were constructed using the TWOPOINT, BUILD and CHROMPIC options of the CRIMAP package²².
To allow utilisation of this package, full-sib families related via the boar or sow were disconnected and treated

related via the boar or sow were disconnected and treated independently. By doing so, some potentially usable information was neglected, yielding, however, unbiased estimates of recombination rates.

QTL mapping: (i) Mapping Mendelian QTL: Conventional QTL mapping was performed using a multipoint maximum likelihood method. The applied model assumed one segregating QTL per

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chromosome, and fixation of alternate QTL alleles in the respective parental lines, Piétrain (P) and Large White (LW). A specific analysis program had to be developed to account for the missing genotypes of the parental generation,

5 resulting in the fact that the parental origin of the F1 chromosomes could not be determined. Using a typical "interval mapping" strategy, an hypothetical QTL was moved along the marker map using user-defined steps. At each position, the likelihood (L) of the pedigree data was computed as:

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$$L = \sum_{\phi=1}^{2'} \prod_{i=1}^{n} \sum_{G=1}^{4} (P(G|M_i, \theta, \phi)P(y_i|G))$$

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P or right chromosme P), there is a total of 2^r combinations for r F1 parents.

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$$\prod_{i=1}^{n} n F2$$

(see above).

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 $\sum_{G=1}^4$ ith F2 offspring, over the four possible QTL genotypes: P/P, P/LW, LW/P and LW/LW

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 $P(G|M_i,\theta,\phi)M_i$: the marker genotype of the *i*th F2 offspring and its F1 parents, (ii): the vector of recombination rates between adjacent markers and between the hypothetical QTL and its flanking markers, and (iii) θ the considered marker-QTL phase combination of the F1 parents.

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Recombination rates and marker linkage phase of F1 parents are assumed to be known when computing this probability. Both were determined using CRIMAP in the map construction phase

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 $P(y_i|G)\,y_i)$ of offspring i, given the QTL genotype under consideration. This probability is computed from the normal density function:

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$$P(y_i|G) = \frac{1}{\sqrt{2\pi}\sigma} e^{\frac{-(y_i - \mu_G)^T}{2\sigma^2}}$$

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g is the phenotypic mean of the considered QTL genotype (PP, PL, LP or LL) and σ^2 the residual variance σ^2 was considered to be the same for the four QTL genotypic classes.

The values of μ_{PP} , $\mu_{PL}=\mu_{LP}$, μ_{LL} and σ^2 maximizing L were determined using the GEMINI optimisation routine²³. The likelihood obtained under this alternative H₁ hypothesis was compared with the likelihood obtained under the null . hypothesis $H_{\mbox{\scriptsize C}}$ of no QTL, in which the phenotypic means of the

four QTL genotypic classes were forced to be identical. The difference between the logarithms of the corresponding likelihoods yields a lodscore measuring the evidence in favour of a QTL at the corresponding map position. (ii) Significance thresholds: Following Lander & Botstein24,

lodscore thresholds (T) associated with a chosen genome-wise significance level, were computed such that:

 $\alpha = (C + 9.21GT)\chi_{2}^{2}(4.6T)$

C corresponds to the number of chromosomes (= 19), Gcorresponds to the length of the genome in Morgans (= 29),

and χ_1^2 (4.6T) denotes one minus the cumulative distribution function of the chi-squared distribution with 2 d.f. Single point 2ln(LR) were assumed to be distributed as a chi-squared distribution with two degrees of freedom, as we were fitting both an additive and dominance component. To account for the

fact that we were analysing multiple traits, significance levels were adjusted by applying a Bonferoni correction corresponding to the effective number of independent traits that were analyzed. This effective number was estimated at 16 following the approach described by Spelman et al. 25.

Altogether, this allowed us to set the lodscore threshold associated with an experiment-wise significance level of 5%

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at 5.8. When attempting to confirm the identified QTL in an independent sample, the same approach was used, however, setting C at 1, G at 25cM and correcting for the analysis of 4.5 independent traits (as only six traits were analyzed in this sample). This yielded a lodscore threshold associated with a Type I error of 5% of 2.

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parent of a given sex would have an effect on phenotype, the QTL alleles transmitted by the other parent being "neutral". The likelihood of the pedigree data under this hypothesis was computed using equation 1. To compute $P(y_i \mid G)$, however, the phenotypic means of the four QTL genotypes were set at $\mu_{PP} = \mu_{PL} = \mu_P$ and $\mu_{LP} = \mu_{LL} = \mu_L$ to test for a QTL for which the paternal allele only is expressed, and $\mu_{PP} = \mu_{LP} = \mu_P$ and $\mu_{PL} = \mu_P$

(iii). Testing for an imprinted QTL: To test for an imprinted QTL, we assumed that only the QTL alleles transmitted by the

 $\mu_{LL}=\mu_L$ to test for a QTL for which the maternal allele only is expressed. It is assumed in this notation that the first subscript refers to the paternal allele, the second subscript to the maternal allele. H_0 was defined as the null-hypothesis of no QTL, H_1 testing the presence of a Mendelian QTL; H_2 testing the presence of a paternally expressed QTL, and H_3

testing the presence of a maternally expressed QTL.

RT-PCR: Total RNA was extracted from skeletal muscle according to Chirgwin et al. 26. RT-PCR was performed using the Gene-Amp RNA PCR Kit (Perkin-Elmer) The PCR products were purified using QiaQuick PCR Purification kit (Qiagen) and sequenced using Dye terminator Cycle Sequencing Ready Reaction (Perkin Elmer) and an ABI373 automatic sequencer.

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In example 2 we report the identification of a QTL with major effect on muscle mass and fat deposition mapping to porcine 2pl.7 The QTL shows clear evidence for parental imprinting strongly suggesting the involvement of the *Igf2* locus.

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A Piétrain X Large White intercross comprising 1125 F_2 offspring was generated as described^{6,7}. The Large White and Piétrain parental breeds differ for a number of economically important phenotypes. Piétrains are famed for their exceptional muscularity and leannes ⁸ (Figure 2), while Large Whites show superior growth performance. Twenty-one distinct phenotypes measuring (i) growth performance (5), (ii) muscularity (6), (iii) fat deposition (6), and (iv) meat quality (4), were recorded on all F_2 offspring.

In order to map QTL underlying the genetic differences between these breeds, we undertook a whole genome scan using microsatellite markers on an initial sample of 677 F2 individuals. Analysis of pig chromosome 2 using a ML multipoint algorithm, revealed highly significant lodscores (up to 20) for six of the 12 phenotypes measuring muscularity and fat deposition at the distal end of the short arm of chromosome 2 (Figure 3a). Positive lodscores were obtained for the remaining six phenotypes, however, not reaching the genome-wise significance threshold (= 5%). To confirm this finding, the remaining sample of 355 F_2 offspring was genotyped for the five most distal 2p markers and QTL analysis performed for the traits yielding the highest lodscores in the first analysis. Lodscores ranged from 2.1 to 7.7, clearly confirming the presence of a major QTL in this region. Table 2 reports the corresponding ML estimates for the three genotypic means as well as the corresponding residual variance.

Bidirectional chromosome painting establishes a correspondence between SSC2p and HSAllpter-q13 $^{9,1\dot{c}}$. At least

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two serious candidate genes map to this region in man: the myogenic basic helix-loop-helix factor, MyoD, maps to HSAllp15.4, while Igf2 maps to HSAllp15.5 MyoD is a well known key regulator of myogenesis and is one of the first myogenic markers to be switched on during development 11. A previously described amplified sequence polymorphism in the porcine MyoD gene¹² proved to segregate in our F_2 material, which was entirely genotyped for this marker. Linkage analysis positioned the MyoD gene in the SW240-SW776 (odds > 1000) interval, therefore well outside the lod-2 drop off support interval for the QTL (figure 1). Igf2 is known to enhance both proliferation and differentiation of myoblasts in vitro13 and to cause a muscular hypertrophy when overexpressed in vivo. Based on a published porcine adult liver cDNA sequence 14 , we designed primer pairs allowing us to amplify the entire Igf2 coding sequence with 222 bp of leader and 280 bp of trailor sequence from adult skeletal muscle cDNA. Piétrain and Large White RT-PCR products were sequenced indicating that the coding sequences was identical in both breeds and with the published sequence. However, a G A transition was found in the leader sequence corresponding to exon 2 in man (Figure 4). We developed a screening test for this single nucleotide polymorphism (SNP) based on the ligation amplification reaction (LAR), allowing us to genotype our pedigree material. Based on these data, Igf2 was shown to colocalize with the SWC9 microsatellite marker (= 0%), therefore located at approximately 2 centimorgan from the most likely position of the QTL and well within the 95% support interval for the QTL (figure 1). Subsequent sequence analysis demonstrated that the microsatellite marker SWC9 is actually located within the 3' UTR of the Igf2 gene. Combined with available comparative mapping data for the PGA and FSH

loci, these results suggest the occurrence of an interstitial

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inversion of a chromosome segment containing MyoD, but not Igf2 which has remained telomeric in both species.

Igf2 therefore appeared as a strong positional allele having the observed QTL effect. In man and mouse, Igf2 is known to be imprinted and to be expressed exclusively from the paternal allele in several tissues 15. Analysis of skeletal muscle cDNA from pigs heterozygous for the SNP and/or SWC9, shows that the same imprinting holds in this tissue in the pig as well (Figure 4). Therefore if Igf2 were responsible for the observed effect, and knowing that only the paternal Igf2 allele is expressed, one can predict that (i) the paternal allele transmitted by F1 boars (P or LW) would have an effect on phenotype of F2 offspring, (ii) the maternal allele transmitted by F1 sows (P or LW) would have no effect on phenotype of F2 offspring, and (iii) the likelihood of the data would be superior under a model of a bimodal (1:1) F2 population sorted by inherited paternal allele when compared to a conventional "Mendelian" model of a trimodal (1:2:1) F2 population. The QTL mapping programs were adapted in order to allow testing of the corresponding hypotheses. H_0 was defined as the null-hypothesis of no QTL, H₁ as testing for the presence of a Mendelian QTL, H₂ as testing for the presence of a paternally expressed QTL, and ${\rm H}_{3}$ as testing for the presence of a maternally expressed QTL.

Figure 3 summarizes the obtained results. Figure 3a, 3b and 3c respectively show the lodscore curves corresponding to $\log_{10} (H_2/H_0)$, $\log_{10} (H_3/H_0)$ and $\log_{10} (H_2/H_1)$. It can be seen that very significant lodscores are obtained when testing for the presence of a paternally expressed QTL, while there is no evidence at all for the segregation of a QTL when studying the chromosomes transmitted by the sows. Also, the hypothesis of a paternally expressed QTL is significantly more likely ($\log_{10} (H_2/H_1) > 3$) than the hypothesis of a "Mendelian" QTL

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for all examined traits. The fact that the same tendency is

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observed for all traits indicates that it is likely the same imprinted gene that is responsible for the effects observed on the different traits. Table 2 reports the ML phenotypic means for the F2 offspring sorted by inherited paternal QTL allele. Note that when performing the analysis under a model of a mendelian QTL, the Piétrain and Large White QTL alleles appeared to behave in an additive fashion, the heterozygous genotype exhibiting a phenotypic mean corresponding exactly to the midpoint between the two homzygous genotypes. This is exactly what one would predict when dealing with an imprinted QTL as halve of the heterozygous offspring are expected to have inherited the P allele from their sire, the other halve the LW allele.

These data therefore confirmed our hypothesis of the involvement of an imprinted gene expressed exclusively from the paternal allele. The fact that the identified chromosomal segment coincides precisely with an imprinted domain documented in man and mice strongly implicates the orthologous region in pigs. At least seven imprinted genes mapping to this domain have been documented (Igf2, Ins2, H19, Mash2, $p57^{\kappa_1p_2}$, K_vLQTL1 and TDAG51) (ref. 15 and Andrew Feinberg, personal communication). Amongst these, only Igf2 and Ins2 are paternally expressed. While we cannot exclude that the observed QTL effect is due to an as of yet unidentified imprinted gene in this region, its reported effects on myogenesis in vitro and in vivo¹³ strongly implicate Igf2. Particularly the muscular hypertrophy observed in transgenic mice overexpressing Igf2 from a muscle specific promotor are in support of this hypothesis (Nadia

Rosenthal, personal communication. Note that allelic variants of the INS VNTR have recently been shown to be associated

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with size at birth in man^{16} , and that the same VNTR has been shown to affect the level of Igf2 expression¹⁷.

The observation of the same QTL effect in a Large White x Wild Boar intercross indicates the existence of a series of at least three distinct functional alleles. Moreover, preliminary evidence based on marker assisted segregation analysis points towards residual segregation at this locus within the Piétrain population (data not shown). The occurrence of an allelic series might be invaluable in identifying the causal polymorphisms which - based on the quantitative nature of the observed effect - are unlikely to be gross gene alterations but rather subtle regulatory mutations.

The effects of the identified QTL on muscle mass and fat deposition are truly major, being of the same magnitude of those reported for the CRC locus^{6,7} though apparently without the associated deleterious effects on meat quality. We estimate that both loci jointly explain close to 50% of the Piétrain versus Large White breed difference for muscularity and leanness. Understanding the parent-of-origin effect characterizing this locus will allow for its optimal use in breeding programs. Indeed, today half of the offspring from commercially popular Piétrain x Large White crossbred boars inherit the unfavourable Large White allele causing considerable loss.

The QTL described in this work is the second example of a gene affecting muscle development in livestock species that exhibits a non-mendelian inheritance pattern. Indeed, we have previously shown that the callipyge locus (related to the qualitative trait wherein muscles are doubled) is characterized by polar overdominance in which only the heterozygous individuals that inherit the CLPG mutation from their sire express the double-muscling phenotype⁵. This

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demonstrates that parent-of-origin effects affecting genes underlying production traits in livestock might be relatively common.

5 Example 3:

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Generating a reference sequence of IGF2 and flanking loci in the pig.

The invention provides an imprinted QTL with major effect on muscle mass mapping to the IGF2 locus in the pig, and use of the QTL as tool in marker assisted selection. To fine tune this tool for marker assisted selection, as well as to further identify a causal mutation, we have further generated a reference sequence encompassing the entire porcine IGF2 sequence as well as that from flanking genes.

To achieve this, we screened a porcine BAC library with IGF2 probes and identified two BACs. BAC-PIGF2-1 proved to contain the INS and IGF2 genes, while BAC-PIGF2-2 proved to contain the IGF2 and H19 genes. The NotI map as well as the relative position of the two BACs is shown in Figure 5. BAC-PIGF2-1 was shotgun sequenced using standard procedures and automatic sequencers. The resulting sequences were assembled using standard software yielding a total of 115 contigs. The corresponding sequences are reported in figure 6. Similarity searches were performed between the porcine contigs and the orthologous sequences in human. Significant homologies were detected for 18 contigs and are reported in Figure 7.

For BAC-PIGF2-2, the 24 Kb NotI fragment not present in BAC-PIGF2-1 was subcloned and sequenced using the E2::TN transposon approach and ABI automatic sequencers. Resulting

sequences were assembled using the Phred-Phrap-Consed program suit, yielding seven distinct contigs (figure 8). The contig sequences were aligned with the corresponding orthologous human sequences using the compare and dotplot programs of the GCG suite. Figure 9 symmarizes the corresponding results.

Example 4: Identification of DNA sequence polymorphisms in the IGF2 and flanking loci.

Based on the reference sequence obtained as described in Example 1, we resequenced part of the IGF2 and flanking loci from genomic DNA isolated from Pietrain, Large White and Wild Boar individuals, allowing identification of DNA sequence polymorphisms such as reported in figure 10.

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Legends to the figures

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Fig. 1: Test statistic curves obtained in QTL analyses of chromosome 2 in a Wild Boar/Large White intercross. The graph plots the F ratio testing the hypothesis of a single QTL at a given position along the chromosome for the traits indicated. The marker map with the distances between markers in Kosambi centiMorgan is given on the X-axis. The horizontal lines represent genome-wise significant (P<0.05) and suggestive levels for the trait lean meat in ham; similar significance thresholds were obtained for the other traits.

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Figure 2: Piétrain pig with characteristic muscular hypertrophy.

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Figure 3: Lodscore curves obtained in a Piétrain x Large White intercross for six phenotypes measuring muscle mass and fat deposition on pig chromosome 2. The most likely positions

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of the Igf2 and MyoD genes determined by linkage analysis with respect to the microsatellite marker map are shown. H_0 was defined as the null-hypothesis of no QTL, H_1 as testing for the presence of a Mendelian QTL, H_2 as testing for the presence of a paternally expressed QTL, and H_3 as testing for

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the presence of a maternally expressed QTL. 3a: $log_{10}(H_1/H_{01}, 3b:log_{10}(H_2/H_0), 3c: log_{10}(H_3/H_0)$

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Figure 4: A. Structure of the human *Igf2* gene according to ref. 17, with aligned porcine adult liver cDNA sequence as reported in ref. 16. The position of the *nt241(G-A)* transition and *Swc9* microsatellite are shown. B. The corresponding markers were used to demonstrate the monoallelic (paternal) expression of *Igf2* in skeletal muscle

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5 and liver of 10-week old fetuses. PCR amplification of the nt421 (G-A) polymorphism and Swc9 microsatellite from genomic 10 DNA clearly shows the heterozygosity of the fetus, while only the paternal allele is detected in liver cDNA (nt421(G-A) and Swc9) and muscle cDNA (Swc9). The absence of RT-PCR product for nt421(G-A) from in fetal muscle points towards the 15 absence of mRNA including exon 2 in this tissue. Parental origin of the foetal alleles was determined from the genotypes of sire and dam (data not shown). 10 20 Figure 5: A NotI restriction map showing the relative position of BAC-PIGF2-1 (comprising INS and IGF2 genes), and BAC-PIGF2-2 (comprising IGF2 and H19 genes). 25 15 Figure 6: Nucleic acid sequences of contig 1 to contig 115 derived from BAC-PIGF2-1 which was shotgun sequenced using standard procedures and automatic sequencers. 30 Figure 7: Similarity between porcine contigs of figure 6 and orthologous sequences in human. 35 Figure 8 Nucleic acid sequences of contig 1 to contig 7 derived from BAC-PIGF2-2, (the 24 Kb NotI fragment not present in BAC-PIGF2-1) which was subcloned and sequenced using the EZ::TN transposon approach and ABI automatic 40 sequencers. Figure 9: Similarity between porcine contigs of figure 8 and orthologous sequences in human. 45 30 Figure 10: DNA sequence polymorphisms in the IGF2 and

White and Wild Boar individuals.

flanking loci from genomic DNA isolated from Piétrain, Large

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5				n=30		- 67.3°	5.02b	70.8°	35.2°		24.7	244	19.7	
10		LP/WM		n≕43			4.94°	69.3	34.5 ^b		25.5 ^b	238	21.8	
15		Least squares means ⁵ WP/WM WP/LM		n=43		64.2	4.72*	66.7	33.0*		27.7*	225*	18.4*	
20	ross'	Least squ		n≕62		63.6	4.69	66.3	31.9		27.2*	226*	18.6	
	White interc	Percent of F,				9	3	4	4		4	4		·
25	oar/Large	Per Per				30.6	24.3	17.4	15.4		10.4	14.4	8.	
30	a Wild B	Map position ³				0	-	0	-		0	. 0		
	omosome 2 ir	Imprinting	0			19.1***	16.8***	**9.6	4 .8*		8.7**	11.4***	•1.9	,
35	sis for pig chr	F ratio ²				24.4***	18.1***	12.2**	10.3**		7.1*	9.7**	5.7	
40	f OTL analy				its S		ım, kg	back, %	area, cm²		depth, mm	<u>tans</u>		4; *** P<0.001
45	Table 1 Summary of OTL analysis for pig chromosome 2 in a Wild Boar/Large White intercross ¹	Trait	$\Gamma_{P/LM}$		Body composition traits	Lean meat in ham, %	Lean meat mass in ham, kg	Lean meat + bone in back, %	Longissimus muscle area, cm²	Fatness traits	Average back fat dep	Weight of internal organs Heart, gram	Meat quality traits Reflectance value, EEL	• P<0.05; •• P<0.01; •
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level.

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10	Table 1	l, continue	d

'Only the traits for which the QTL peak was in the IGF2 5 region (0-10 cM) and the test statistic reached the nominal significance threshold of F=3.9 are included. 2"QTL" is the test statistic for the presence of a QTL under a genetic model with additive, dominance, and imprinting effects (3 d.f.) while "Imprinting" is the test statistic for the presence of an imprinting effect (1 d.f.), both obtained at the position of the QTL peak. Genome-wise significance thresholds, estimated by permutation, were used for the QTL test while nominal significance thresholds were used for the Imprinting test. 3 In cM from the distal end of 2p; IGF2 is located at 0.3 cM. 'The reduction in the residual variance of the F, population effected by inclusion of an imprinted QTL at the given position. 5 Means and standard errors estimated at the IGF2 locus by 20 classifying the genotypes according to the population and parent of origin of each allele. W and L represent alleles derived from the Wild Boar and Large White founders, respectively; superscript P and M represent a paternal and maternal origin, respectively. Figures with different letters (superscript a or b) are significantly different at least at

the 5% level, most of them are different at the 1% or 0.1%

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Table 2 Maximum likelihood phenotypic means for the different F2 genotypes estimated under (i) a model of a mendelian QTL, and (ii) a model assuming an imprinted QTL.

	Mendeli	an QTL	Imprinted QTL				
Traits							
	րլա/ւա	րլա/ը	µ₽/₽	R	PPAT/LW	UPAT/P	R
BFT (cm)	2.98	2.84	2.64	0.27	2.94	2.70	0.27
% ham	21.10	21.56	22.15	0.83	21.23	21.9	0.83
						5	
% loin	24.96	25.53	26.46	0.91	25.12	26.1	0.93
,						4	
% lean	65.02	65.96	67.60	1.65	65.23	67.0	1.67
cuts						5	
g _e	6.56	6.02	5.33	0.85	6.43	5.56	0.85
backfat							
% fat	28.92	27.68	26.66	1.46	28.54	26.9	1.49
cuts						9	

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Claims

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CLAIMS

1. A method for selecting a domestic animal for naving desired genotypic properties comprising testing said animal for the presence of a parentally imprinted quantitative trait locus (QTL).

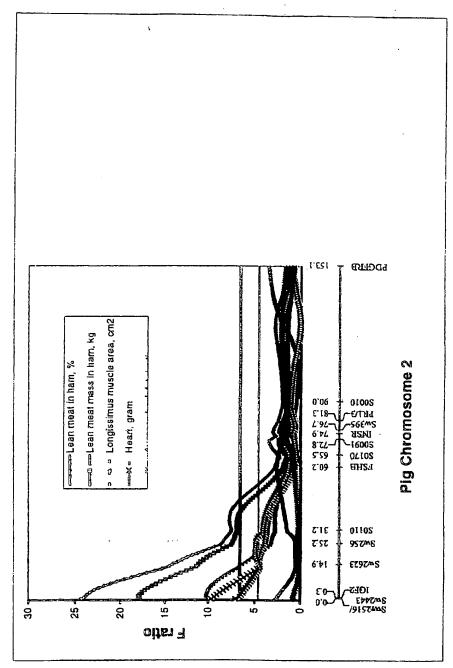
- 2. A method according to claim 1 further comprising testing a nucleic acid sample from said animal for the presence of a parentally imprinted quantitative trait locus (QTL).
 - 3. A method according to claim 1 or 2 wherein in the pig said QTL is located at chromosome 2.
- 4. A method according to claim 2 or 3 wherein said QTL is mapping at around position 2p1.7.
 - 5. A method according to claim 1 to 4 wherein said QTL is related to the potential muscle mass and/or fat deposition cf said animal.
- 6. A method according to claim 5 wherein said QTL comprises at least a part of an insulin-like growth factor-2 (IGF2) gene.
 - 7. A method according to anyone of claims 1 to 6 wherein in the pig said QTL comprises a marker characterised as nt241(G-
- 20 A) or as Swc9, as identified in figure 4.
 - 8. A method according to anyone of claims 1-7 wherein a paternal allele of said QTL is predominantly expressed in said animal.
- 9. A method according to anyone of claims 1-7 wherein a
- 25 maternal allele of said QTL is predominantly expressed in said animal.
 - 10. An isolated and/or recombinant nucleic acid comprising a parentally imprinted quantitative trait locus (QTL) or functional fragment derived thereof.
- 30 11. An isolated and/or recombinant nucleic acid comprising a synthetic parentally imprinted quantitative trait locus (QTL)

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10		derived from at least one chromosome or functional fragment derived thereof.
		12. A nucleic acid according to claim 10 or 11 at least
		partly derived from a Sus scrofa chromosome.
	5	13. A nucleic acid according to claim 12 wherein said nucleic
15		acid is at least partly derived from a Sus scrofa chromosome
		2, preferably from a region mapping at around position 2p1.7.
	•	14. A nucleic acid according to any one of claims 10 to 13
		wherein said QTL is related to the potential muscle mass
20	10	and/or fat deposition of said animal.
20		15. A nucleic acid according to any one of claims 10 to 14
		wherein said QTL comprises at least a part of a insulin-like
		growth factor-2 (IGF2) gene.
25		16. A nucleic acid according to anyone of claims 10 to 15
25	15	wherein a paternal allele of said QTL is capable of being
	• •	predominantly expressed.
		17. A nucleic acid according to anyone of claims 10 to 16
		wherein a maternal allele of said QTL is capable of being
30		predominantly expressed.
	20	18. Use of a nucleic acid or fragment derived thereof
		according to claim 10 in a method according to anyone of
		claims 1-9.
35		19. Use according to claim 18 to select a breeding animal or
		animal destined for slaughter for having desired genotypic or
	25	potential phenotypic properties.
		20. Use according to claim 19 wherein said properties are
40		related to muscle mass and/or fat deposition.
		21. An animal such as pig selected by a use according to
	•	claim 18 to 20.
	30	22. A animal according to claim 21 characterised in being
45		homozygous for an allele at a paternally imprinted QTL,
		preferably located at a Sus scrofa chromosome 2 mapping at
		around position 2p1.7.
		23. An animal according to claim 21 or 22 wherein said QTL is
50	35	related to the potential muscle mass and/or fat deposition of

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10		said pig and/or wherein said QTL compa insulin-like growth factor-2 (IGF2) 24. A transgenic animal comprising a to anyone of claims 11 to 16.) allele.
15	5	25. An animal according to anyone of male.26. Sperm or an embryo derived from a anyone of claims 21-25.	
20	10	27. Use of a sperm or an embryo according animals destined for slaught	
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		•	





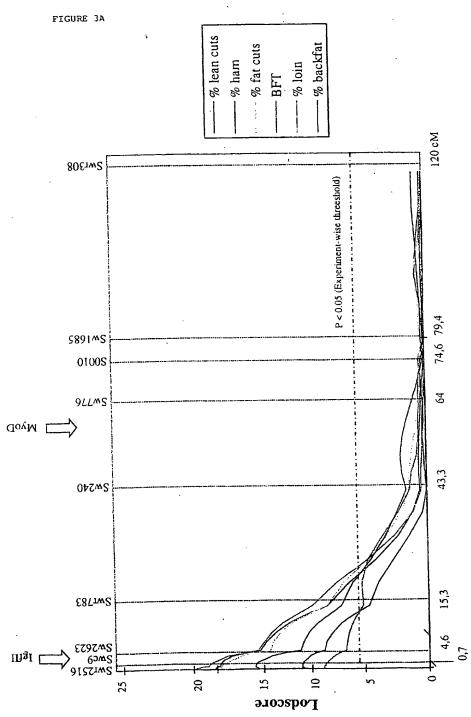
SUBSTITUTE SHEET (RULE 26)

. 4

FIGURE 2

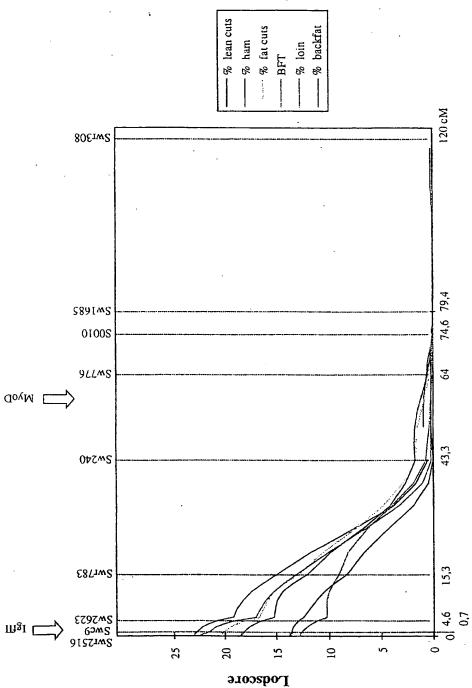


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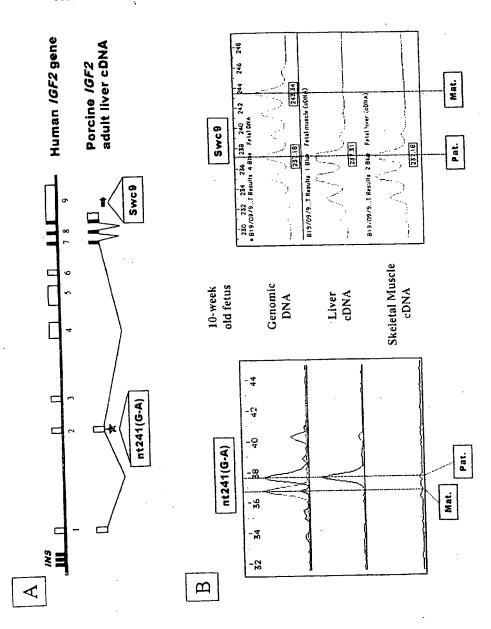
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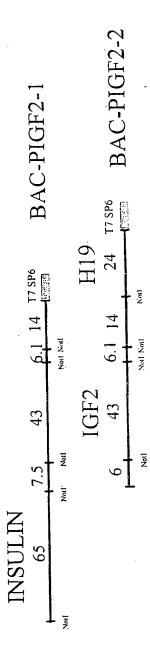
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FIGURE 4



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FIGURE 5



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FIGURE 6

Contig 1 (500 bp)

Contig 2 (943 bp)

Contig 3 (1500 bp)

TGGGCCCTTTCCACACACCTCTGGCTGGGCCCTG
GGGGACCCTTUTGGGGGGATGTGGGTGCACAGCCAGGGCCACCAGGACACCGGGGCTCCCTTCCC
TCGGGTCCCTGAGACCCCTGGCCTCCCGCCAGCACTCCCTGCCGAGGAGCCCGAGATCCGGGCCTTTCACACCCGACCCAGACCCAGACCCGTCTACACCCGTCTCTACTAGCAGTTCAGAGTTTCAGT
GACGCCAAGGACAAGCTCAGGTGGGGCCGGGGCCCCCAAACTGGAGGATCCAGCCTGCAGCCCGGC
TATGAGCCCATTTCCCAGCAGGAGGAGCTGCTGCGGACCCACACCCGTCACAACCCCCCTCCCACAGCTGGAACC
CCAGAAAGCCTGCGGGGGGGACCTGCAGGGCTG

Contig 4 (3024 bp)

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FIGURE 6. CONTD.

AGGCGGCTCCAGGGAGGAATCTTACGGAGTCAAGGCCCGGGTGCCGCTGGTCTCCGAGTGACATCGCCGTGGT GTCCCRTCTGCCGGCCCACATGCCCGTGAGAGAWGCCCCATCCCCCTGGGAGGGGCCCCGTGCCGGGCAGGC GGCGGGAGGCCCAGGACCGGTGCTGCTGCGGCTTCCACTCCAGGCTGGGCGGGGTGGGGGGTGGCTGTCTCT GTGTGACCGGCTCTCCCCUCAGCAGGTGCCGTGGAGCTGGGCGGAGGCCTGGGCGGCCTGCAGGCCCTGGGGG TGGAGGGGCCCCCCAGAAGCGTGGCATCGTGGAGCAGTGCTGCACCAGCATCTGTTCCCTCTACCAGCTGGA GAACTACTGCAACTAGGCCGCCCTGAGGGCGCCCTGCTGCTCCCCGCACCCCAAAACCCAATAAAGTCCTGAA TGACCCCGGCCGAGTCCTGTGTCTGTGTGGCCTGGGGCGGGGCCCTGGTGGGGGAGGGGCCAGAAGGCTGT GGGGGGCCTGCCTGCGCCCCCTCTCTGCTCTCGCCACATCCCCTGCTCTAAGCTTCCTCCACATGCATCGGGT GCCCACAGGCACATGCCCACCGGGGGACCAGGGCCCAGGGCAGGGCCCTTCAATGTGGCGAGCTCTUUTTTTC AGACTGCTCTTGGGCACACAAAATAGCCCAGGGGCTTCTTGGGCCTGCTGCTGTCTGGGAGGTCAGAGAGTGA CCCCGCGGGACCAAGACUTGGCCAGGUTGCCAGTCGCCCAGGCCAAACCAATCTGCACCTTTGCTGAAGGTTU CACCCGGCCAGCACTGGGGGCGGCCGGGCCTAGAGCTGGGCCCCGGGCCCCAGGGACTGCACACCCGCCAG AGGTGGGCCTGAGGGGTGGCAGCAGGCTCTCCCCCTGGGACCCAGCTGGGCAGCTCACCTCTCAACACG AGGCTCTCACCTGTGTCUTUUCCTCCCCACGGCCACACAGACACCCCTGGGGAGAAGTCACAGGCCCUCAGCA GGCCCCGCCCTG(AGAGAGGAGCCAGGGCTGGCAGGCGGTGGCCGGACACTGGACCCGGAAGGGGGG GACTCCTTFGGGACTCGGGGCCCCTGAGCCGCCCCCACTCGCAGGACTCACCCGGTGTGCGGTCCTGGGTGAG TGGGGGCTTGGGAGAGGGTCACTCTTGTCCCTCGGGTGGGGAAGGCTGAGAGTCATGGTGTGACAGCGCCCTC GGCCTGCCGGGTGGGGGGTCTCTCCCTTCTCCCGAGCCCAGATCCCCGGGTAC

Contig 5 (1730 bp)

ACCAGGCAAGGTGGTCCGAGCGGTCATTCACAGACAGAACCAGCAGAGGGGGCCCAAAGCCCCCTTTTGACAA
ACTCCCCTTCGCCCTGAGCCGAAAGTCCAGGGGGCACGTGGACCTCTCTGCAGGGCTCTGCCACCCCTGCTGC
CGCTTGCCAGCACTCACAGCGGCTGCGGGGGTGCCCAACAGGCCGGCTACCCTGAGCTCTGAGGCGATGGA
CTTTAGCAGGGAACGAGGGGACTCCTGGGGGTGACTTTCTTCAGCGCCCACATTGCGGCCCAGCAAACCGAGG
CTGGAGGAGGCCCCTGTGCCCAGCTGGAGCCTTTCCTCAGGGTCTCCAAGGCCTGGGGAAATTGAGGC
TGGGGGCTGGGGGTGTCACTGTCGGGCCAGGAGG

Contig 6 (4833 bp)

ATGTEÄGCTGCACAGCÑTGAGCCCTCGGCCCACTGCTGTGGCCTTGCGGACATTGAGGTGTGTGCCGCCCAG GCGACCACCCTGGCCTCTCAGGGTGCCCGTACAGAGGCGGCTGGGTCCTANGAGGTGCGGGGCTTGGGG ACCGCTGGTGAGTTCAGGACGGGGGTCATGCCACCTCCTTCTGAAGGTTTGGTGAGGTGGCCCTTCTTTAT CGTGATGACAATACTGATTTCTGGAAGAGCCAGGTGTTTTCTGAGGCTGTGGTTGCACTTCTCACGTGGCCA CAAGGTGCCGGGCTCAGATTTGAGAAGCCCTGCGGGAGCGGTGTCATGCGCCAGATTCAGCTTGCCT

FIGURE 6. CONTO.

CCTGCGGGTCTGGGGTCAGGACGTGGTCCCCAGCAGTCTGCTCCAGAGCCTGTCAGTGATGTGTGGGGATTTTA CCGCTAGAACACAGTTTCCTCTGATTCTCAGAAACCAGCAGATGCTTTAGGAGGGGGCGTGCAGGTTTCACCTG TGCTGCANNGCCCCCTGCCACCTGGTCGGAGCCNCAAGACGGCATCTAAAGATCAGTTCCTCATCATCAGTTC CGCAGTGCTGGGGTGGGGGCAGATGAGAACCTCAGGGCTGGGCGCAGAGGTGGGGAGCCCGGACCCCGA CACTGCAGGGGGGCCTCCCCCTTGTAGGAAGAACAATGTCGCTTTGCCACCCAGCCCTCTCCCCAGGGTGCCC CGAACTGTTGCTCCTAAGACCTCTGGGCTGTGTGCTGTAATTCTATAAGTGGCCACCAGGTGTCAGCAGGAGG CCACTTAAGCATCCATGTGGCGGAAACCTGGAGCTGGGGGTTCCTAAGGGTCCCTCGAGTGTCTCCTGAATAA ATAGGCGCTGACCTGATCCCCAGGAAGGGATAACCCTCTCCCAGGCCTAAGAGGCAGTGGGGCAATGAGGTTT ATGTGTCCACTGTACCCCCAAATTGTCTCTTCCTTCCCTCTACCCTGTGTCCCCACCGTGGACGATACACGGA GTGCGAGGCTGCGGGTCACAGCCCTCACAGCCCCAAAGCTGCAGGTCCTGCCTCAGGGGCACCGCAGCTTGGC TGGTCCCCCTTGGGTCCTCCCCACCCTGACCCGTCCTCTGCTCCCCTTTGCTTAAATGCTCTGCGTTTC AAGGTTCTGATGGAATAAAATAGCCCTGCACTGGTGTGTTCCTCTTTGGGGGCTGTGCCAGAAGTGGGAATTCA GACCAGGGCAGAGCTCAGATTCCACATACTGTGTTAGGGATGGCAGGTGCCACATTTCCAGGAGTTTCATTGG TGGTTTGTAAATGCTACTTCCGTTTCAGCCCCTCAGCTGCCCACCTCCAATTTAGGGACCCCCCTTTGG CGGGTTGCCCATGGAACCACATCATCTGGCGTGGGGTGAGCCCTTTATCCTCCCTGGCCCCACTGGGAGGGTT TGGGGAAGTCCCAGCTAAATTTCTCCGTAGGGACCTGGAAGGAGCCCTTGTGACATCTGGGCACAGATAAGAG GTAGGGGGCACAGGCCGTGAACACTTGAAGCTGCAGAGCCCAGAGCAGGAGCCAGGAGCAAGTGACTGCTC CCCACCCAAGAACTGTGGGCTGCGTCACACACTCCCCACTGTGTGCCCTGGACCTGACAGGGCCTTTAGCCT CCCTGCATCCCCCACCCAAGAACCCAGTGAGGGACCCCACTTGCCCCTCCTTAGTGTTGTTATGGCTCTG GGGCATCTGCATTTTGTTTAGGACACCCCCAGCTAGATTTAAGTCCCCCCAAGTGTGACTCTTTCCTCCACTG AAAACCCTGTCCTCCCACCAAAGGGCCCTATCCCTTTAGCTGAGCCAAGGAAATTCAGGAGCGGCCTTGAATG GGGGTGCAGTGAAGGTAGCCGCTCGTGGCCTTCTGGAAACTACATGTGACTTTGCCATTAGGTGAGTCTTTGC TTTGCCCCTGCTCTATCTGCAGGCTTATGGAAGAAGTTTAAATTCCCAGGGACACTTGGTCTAACCAGGCAGC GCTTGTATCTGGGCCCTTCCCCAGCTGCTGACCACCTCTGAGTCTGCGCCTTAGTTGGAGTTTTGGCCAAGCTC AGTATTGTCACTGTCCGGCACCACACACACACGTGCAGGGGGGGTGGTATCAGGTGCCACTGGGGAAGGGACAAAA CTCCCAGGTGAGTCCCCTGCCTCTGGAAGCAAGATGCACATGACCGCACTGTGTTGCAGCTGCATTGGGAGGC CCCGAAGAAGATTTTCTGATCTTTCTCGAACCCTGCTTTTCCCCATCATGCCCCGCCCCCATTTTACCCGT GCCACGCCCACTGGTGTGCCGGGGTGTCAACTGACTGACAAGTGTCAATCTACTGAGGCCCTGCCCAGTCTCC ACCCCCCCACATAGTCCCACCTCCCAGCTGGCAGGGGAGAACTTCCAGCTAATGCCCATGCCCACAATGTCTT TCTGTCAGCCTAGAGCTGCACCAAATCTCCACCCTGTAACATGCTGTGCCCTGGCGTGGGAAGGTGCCAGAGC CAGTTGCCCCAGCAGCCCCAGAACCACTAAGTTUGCACAAAGCTACCCAAATTTGGAGGGGCTTGGGGAAGGG CATGGAGGGGATGAGGGGGGGAAAACTAATTTCAGTTAGCATTTGAGCAGGTGCCACGCTCAGCGTG GAGAGGCTCTCTTGCTTCTAGGGACCCATTATGATGCACACGCTAAAAGCGCCCTTCACCATCTCTCCAGCCT CAGCTTTGTCCCCCTCCTCCTCAGCGGCAACUUGGCTGGAGGGTCTGGCCACTACAGCCAGAGCGCCCC TACTTTGGTGGCGACTGCTACTATTGGCCCAACCAGCGGATCACCGGCCAGGCAGTTTCGGCAGAGAGTCTGG GGCACCAGTGACTCCCCCCTCTTTATCCACCACCCAGGAGCTTCAGGGGACTACACAGCGACTAGAGGGCA GGTAACTGGTCTGCCCTCCCTAGGGCTGCCCCCTCAGAGTGTGTGAGAAAAGCTGCATTGAGTGTTTGGGTGC AGGTGGGCTGGGGGCTTGGGGCAGCCAACAGGAACGGCCCGGACCTCTGCTTCCAGAGGACCCCAGATCCTGGC AAGCTTCGACTTTGGAGGGGACACGAAAGACAGGTGGAGAGGGGACACTTCCCTCTTCTGTACAGACGCCCAC CCGGAGCCACAGAGGCTTTTGCAAGGAAAATAGGTTTTCCCTCACTAATGCAGCAGGCAAAATGGGAGGGCA GGGCTGGAGGGTAGTGCCCCCGCCCCCAGCAGGAGGGCACAGCTCTTTCTGCAAATGTAAAAAAGCAGGGTTT TTCTGTGTGAGAAGTTCCCTCTTGCTGCATGTCCCCACCCCGCCACCAAAGACAACAGGACACTGTGCAGA GGGGCCAGAGCCCCGAGATTTTGGAGTTGTTTTTATATGCATATATACCATTTTGAAAGCAAAGCTTCCCTCT CCCCTACTCCCTACATGTCCCCCTTCACCAAAAAATCCCACCACGTAACTGGAAAGGGGAGTGAGAAGGACGA CGAAGGGGCACTGTCCCCTCCCGTCCCACAGCGGGACTTAAAACGTACAGCTTTTCGCCTCCGGACAGTGTGC CGCCCCTGGCCCCGGTCACGCTCCCCTGCCCGGGGGGCTGAGTGTGGGGGCCAGGGCCTGTCTCCAGGCATGC ATTATTTTGTGCATGAAGGTTTTGTCCCGCCCACCCAGGCTGGTGTTGGGGGGGAAGGGGTTCATTGCTCCAAA GAAGCCCATCTCCCCCCTCAGCCACCTTCAGCCGCCTTCGCAAGGCAGAGCTGTCTCCTCTGCTGTGTGCCTG CCCCGAGGCAGGCATTTGTGTGCGGCCCCCAGCCCCAGGCCCAGGCAGATGGGCCAGCCTGCCCGACAGA CTGTATTTAAAGAAGAAGGAGCTGGGGGGGCCAGAGGCACAGGGGAGCCACGGCCCCAGGTCTGACAAGAT ACACTCTTTATTTTCCCCAGGGGCCGAAGAGTCACCCCTGAACTTGAGGACGAGCAGCCGGATTCCAGCCCCC AGCCCCAGGGCCCCACATCTCCTCGGGCTCAGCCGCGCGCCCAGCTGCCCCCCAGCCTGAGCTGCAGCAGGC AACTGAGGCGGGCAGCCCAGCCCACAGCGGTGAGTGTCTCCAGGACCCCAGGGCAGGGCCCGGTGTCCCC CGGCACAGAGAGCTGTGCTGCAGGCCCAGACCTCCCAGGCCGTTTTAGTTCCCCATCTCCCCTTGGGGGAGGGG TGGGGCTCAGAGGGGCTGGGGTGCATCCGCAGAGCTGGGGTGCAGGGGCTCCAGGTGCCTCTCTCCCAGGCGGC TGGCCCGGAGGGGG

Contig 7 (2014 bp)

ACACCGGTTCTTCAGGACCCACCGCCTAGACGGTCTTAATCCCTTTTCCCCCAGACCTAGATTCCCntig 8 (371 bp)

Contig 9 (2415 bp)

GGCGCTSCGCCTGCTCGGGCAGGTGGAGGCTTCACGCCGGGCCCGCGCCCAGGGACGACCCCTTACCCCGCAG GTCCCAGCGGGACTCGGGCCCCCGGATCCAGCGCTCTAGCCACCTGTGCCCCCACCGCCGCGCGAGGGCTTGTGA CACCTACCACCCTGGCCGCCCCGCGTCCCCCGCGCACGAATCTAGGGATCCTGACACCCCGGAACCTAAGAC GGGGCCCCCATACACTTTCGTACAGCGATTCGGGATTTCTCTCGAACTCTGCAGATCTGTATGGCAAAGTTGA TGGCCTGCATTATTTTTCTGATAATTCAGCGAAAGATGGCGACCAGAGCTATGCGCGTCTGGGTTTTAAAGGC GAAACCCAAATTAACGATCTGGTCAACGAACAGAT

ACAGCATACGTTTTT

Contig 10 (3753 bp)

AGATTCCAATGGGGATCCCGATGAGGAAGCCGCTGCTCGTCCTGCTCGTCTTCTTCGCCTTGGCCTCGTGCTG CTATGCTGCTTACCGCCCCAGTGAGACTCTGTGCGGCGGGGAGCTGGTGGACACCCTCCAGTTTGTCTGCGGG GAGGACCTCTCCCCGAGGGTCTGAGACTTCAGAGCGGGGGGCGCCCTGGCCCTGCGCAGTGATTGGCACCTGC CATGTGCCTGGCTGGGGCTCACACCCCCTGACGTTCCTGCAGCGTGACTCGAAACGGUAAACCGAAGGGACGG AGGCCCCACAGGATGACAGCCTGTCCCCTCCTGCTCCTTGACCTGCCCAGGCCAGGGCTGCAGGCACTG ACATTCACCCATGGTATTGTGGTGCCTGACGTCTTGGCAGTGGGCATGGGTTCATGGACTGTTGGATTGAAAG TGGAATAAGATGGGTTGAAAACCAATAAGAATAAAGCCCUUTGTGGCTGGCGGCATCTGCGAGAGGTGACCCC CACCTCCCAGCAGGCTGGGCCTCAGTCTCCTTACCTGTAGGATCCCTCAGGGGGCGTCCTGGAGAGAGTCCTCG GGACAATGGGGAGGCTGGGGGGCAGGCCCAGCCTGACCCTGAAGGTGGGAGTGTGTGCTCCCCCTGGGCTCAGC CAGCCGCGCTTGGGGCCGGGACCGGGGGGGGCGACGTGGCTCGGGCAAGTTGTCAAGGGCCGCGAGGCTCACCC COGCCONTOGOTOCCCATGTGGCAGCCTCTTCTGCAGCCTCTACTTACCCAUCCTCTGAAATGGGCTGAAAAC ACCCATCTTGGCATGCCAAAGCTTCTCTGTAAAAAGCGTTGCTGCTTCTTGATGCTTC"GAGGCCCCTGCCTG CCCTGGCCTCTGAGCCCTCTCTCTCCCGCCTCGTTTGGGGGCACGGAGTGGCACCATAGAATCTGGCGCTGGG CCTGGGGAGCGGCCCCCTCGTGCCAGGCTTCCCCGAAAGGAGGGCTGGGCTGAGCTCCCGACCCTCTGGACCC CCTTGCAGCCGAGTCGGAGCTGTCCGAGGCGAGGGCGAGGACGGGAAGAGAGGAGGGGGGTGGTTTCTGCTGGT CCTCACTCCTCCTCCGGGTCTTCCTCCTCCTCCATTCCCACCTGTGTCTCCGGGGTCCCGGGGCCGCAG GCTGCCCAGGCGCCTGCTGATCCATTGGGGACCGCACTCGGGTCCCCGCTGGCCTTCGGGTCAGGGCCACGGC CCAAGAGGCCAGGGGCTGTTCCCGAGCAGCCGGCTCACCCCGGCTGTCCCCTTCCCTTCCCCGGGG CGTCCTCTCTCGAGGCCGGGGGCGGGGGGGGCGGGCGGGGCCCCTGTGCTGACGTGC CCTCCCCTTGGTCCTGTGGGACTTCCAGGCAGGCCGGCAAGCCGCTGAACCGCCGCAGCCGTGGCATCGTGG AAGAGTGCTGCCTAGCTGCGACCTGGCCCTGCTGGAGACCTACTGCGCCACCCCCGCCAAGTCCGAGAG GGCTGTCTCCTCTGAGCCGGGGGACCGGGGCCCAGCCGGCTCTTGGGCTTCAAGTGCTGCCAGAGGGGCCTTC CAGAGGITGTTGTTCTGGGACAGGGGCTGGGGGCCAGGCCCCCCCCTGACGGCCCTTCCCCTCTCAGGACA ACTTCCCCAGATACCCCGTGGGCAAGTTCTTCCGCTATGACACCTGGAAGCAGTCCGCCCAACGCCTGCGCAG AAGCGTCACCGACCCCTGACCGCCGTCCCACCGGAGACCCCGCCGCCCACGGGGGCCCCCTCTCCCGAGGCGT CCGGCCATCGGAAGTGAGCCAAATTGTCGTAATTCTGCGGTGCCACCATCCACCTCGTGACCTCCTCTCGACC GGGACCGCTTCCATCAGGTCCCCCTTCTGAGATCTCTGTACCCTTCTCTCTGCGGGCATCTCCGCCCGGGCC CCGTGCCCAACCTCCCATGTCAGGCTAGTCTCCTCCGGCCCCTTCCATCGGGCCGAGGGCATCCAAACCA CAAACUCAATTGGCTTGGTCTGTATCTCCCCCCAAATTNTGCCCCCAATTATCCCCAAGTTACATACCAAAAA TTGAACCCCTCAACCACACCCACATACAATCAGCCCCCGTAAAACGAATTGGCATCTTTAAAACACCAGAAAA AATTGGCTGTGACCCATCATCCAAGAGAAAGGAAGGGACCAAAATTTGCAGGTAGGCTTGTCGCCGCTCACAG CCATCTCCCTCCTGCCACACCCTCGCCGGCCACTGGCGGTGTGGCACCAAGGACCCAGTCCCGTCCTCTC TCTAGTCCCATGACCGAGACCGCGGTGGAGTTGGCTGGGAGACCCCGTGAGATCAGAGGAGGGGAGCACGGAA CCAGAAACCCAAACCTGCACAGGTACAACATGACTGGCCCCCCGCACAGCCCAAACCTCTCATCTCAGTCTC

Contig 19 (500 bp)

Contig 22 (450 bp)

Contig 24 (868 bp)

CAGGACGCANGTGGGCCTGTCTGAGTCCGTCTACACGTCCAGCCAAGGGC
GGCCGCGACCGGCCAGGGTGGGCAGCCCCAGCCTCAGCAGGGCGCTCTCT
GGGGCTCAGGCTGCGCCGACGGGAGTGAGGGTGAGGCGCACTCTGGGG
CTGCTGCCGAGAACCTCGCCCAGCTGCAGCTGGCACACAGGAGACCTG
GGGTGCCTGGTCAGGGTCGTAGTTTGTGGGCAGAACCTCCTCAG
CGCGTCCTTGCATGGGGTCCTAATCACGGAGTAAGGAGCCAGAGAATGAG
GCACGGAGTATCCAGTGTTAACCCTGGATTATGAGAACGGGAGAACTAAT
TGTGGAGCATGGCTCTAAGGAATGAGTATTCGTCACGGAGAACGCGGGG
CCGGTGAATACGGAGAGCGCGTACGACAACGGGGAACGGGGTATCCG
AAGGGGAGGATGGACTATCGGCCGGAGGATGGACACTAGAGGA
TGTATANNGGGCGTCAAT

Contig 25 (500 bp)

ACCAGITTCGATGAGCAATCCCAGCGGGTAACATTATGGCTGCAGCCTG
GTCAATGCCGGTGGAGTTTGAACCTCCACGCGTGGCGATTCTGGTAGATA
AATCGACATGGACCAGGGGGTTGATGAACATAACGGTAAATTTGGCATC
GTTATCCCGGGCGTTGCAGCAACTAACTGGACGGTGGGCGGTGGGAAGTGT
GTCGGGGGGTGATGAAGATAAATTTAATTGCTATGGCATTCCGGTTGTGA
GAGGCCCGGTATTTGGTTTGCCTCTGGTCGAGGAAAAATGTCTGGCGTGG
ATGGAGTGTCGATTTGCTACCTGCGACTTCTGCCAAGAAGAATACGACAC
GCTGTTTTGGCGAAGTAGTATCAGCAGCGCAGACGCACGGGTATTTCTCC
AAGGCCGCTGGCAGTTTGTTGATGATAAGCTCAATACGTTGCATCATTTA
GGTGCTGGGACGTTTCTTACCACCGCCCAAGCGTTTACGGCGGGTTAAGC
Contig 26 (900 bp)

ATGTTTGATGTCCGCGCGTGCTGTAAMATTTACGCTGCTCGCGTTCTTT GGCTTCGTCCACCACCGGAAAACGGACAAAATTTCCGTCATACCTTTTT CTTTCAGGCGGAAGCCAATGTCGTAATCTTCAGTAAGACTCTGCACGTCG AAAGCAATACCGTCACCGTCAGCTAACAGTGCGGTCACGGCGGCGGCT GAAACAGGTGCCGACGCCTGCGCTGGGCACTTGTCCGGCGAGGGCTTCAC GCACCGGAACATCTTTGCCATGCAGCTCTGAAAACTCATCAATGTAAGTC ATGCTGGTGAAGTGCGTCCATTCGCGTTCGAACGGATACACCGGGATCTC AATCAGATCTTTACGCTCGACCAGATAGTTGAACAGACGCAATTCCATCG GTGAAATCACATCTTCGGCGTCATGCAGAATAAAACCACCAAAAGCGAAA TTGGCGCTACGCTCAAATTGGGTGATGGCGTCCAGCACGTTGTTCAGACA GTCGGCTTTGCTGGTGGGGCCAGGACGCGCGCAGACTACCTTATGCACAT TCGGGAAGCGAGCGCACACTTCGTCAACATCACGCTGAGTATCGGGGTCG TTGGGGTAGGTGCCAACAAGATATGATAGTTTTCGTAGTCGAGCGTGGT CGCCGCCAGCTCGGCCATATTGCCGATGACGCCGTTTCATTCCACGCCG GAACCATAATCGCTAACGUTT"TTCATCTGGTT"ATACAGTTCGCGGTAA CTCATTCGCGGGTAGCGGCGATAAACACTCAACTTGCGTTTAATGCGGCG TACCCAGTATACGACATCTATAAAAAAAATCGTCCAGCCCGCTGATGAACA TGATGACCGCTAACGTTATCGCGATTACTTTTAAGCCGTATAGCCAGGTA Contig 27 (500 bp)

AGCTGATGCCCCCAGCTGTGGTCCCTTCCCTTCCGTCAGGGCAGGTTCT
GTCCCTCTTGCAGCCACCGTCACTGCTGTGGACAGGGCTGCCACCACCCCC
GTCCACCAAGAGCGTCCCTGGCCACGGGCCGGCCCGCTCCTGACGCA
CCATGTGTTCAAGGCAAGAGCACTGGACAGAGGGTCCAGACGTCCCTTTC
TCCTGCTCAGGCCTGGGCGGGGCAGCCCTGGCGGAAGAGGCCCTGGGCA
TCAGAGCCTTGTGGCCTGAGCTTGGCGCCCTCCCCACCTCCGT
CCTGCTCCTCGCCGCCCTCACCGACCTTCCCGGCCCCCAGCCTCATT
ACTCTTAAGGACCTTAGCCCCCTATGCTGAAATGCTGTACCTCGTGCTTG
TTTTCATCTGTTTATTACCTTATCTTCATTCTTCGTTGTTTATAGGACACCTCT
Contig 28 (450 bp)

AGTGCGGTCGGGCCGTCCTGACGCTCAACACCGTATTTCCACGCGACCGC
GGATTCAACCTGGTCACACGGACGCCATGTAGACATGTTCGGGGTTACGC
GCAGAGAAGCGACCTCCACCGCTGGTGGAGTCGGGCCGTCTTCGCCC
AGACCAGTGGAGTCGTGGGTGTAAACCATCACCTGACGCTGTTTCATCAG
CGCAGCCATACGTACGGCGTTACGTGCTATTCCACGAACATCAGGAAGG
TGGAGGTGTACGGCAGGAAGCCACCGTGCAGGAGATACCGTTAGCAATC
GCGGTCATACCGAACTCGCGAACCACCGTAGTGGATGTAGTTACCCGCAGC
ATCTTCGTTGATTGCTTTAGAACCAGACCACAGGGTCAGGTTAGACGGCC
CCGGGTCAGCAGAACCCCCGAGGAATTCCGGCAACACGCCGGACGAACGCT
Contig 29 (450/bp)

TCAGGCCAATCTGTCTGGTCTCCAATGGGGACAATTTGGTTCTTTAGGCT
TCTGTCCAATGGTCCGAATGGCCCACTCCCCGGGCGCCGAAGGGTCC
TCTGTGCCTCGGGTGGGCAGGAAGCTCCCCGGGCCCCCAGGGTCGTGCCAGCC
CCGTCACCGGGGCCAGAAGCTTCGGGCCTTAGCTGGCTAGTCGGGCTG
CTGTGCAGGGGGCTGGGGGCAGAGGCGGGGGTAAACCTC
CCAGCCGCCCGGGTCCCTGCCCAGCCCTAGGCGCCGAGACGGTGACCCCAGCTG
GGTCGGTACCGCAGCCCTAGGCCCCAGGTGACCCCAGCTG
TCGCACACGCTCGCAGCCTCTCTTGCTCATCAGGGCTCATCCCTCTGGACC
CCACTCTCACCTCCCCCCCCTGACCCCATGAAGCCCCGCGGA
CONLIG 30 (600 bp)

GCGCGGGCGTTCCGGCTGGGTATTTAACGTGGTCACCGCTTCGGCGGC GCGGTCGGTAACCAACTGACCAGTAACCCGCTGGTGCGCAAACTGTCGTT TACCGGTTCGACCGAAATTGGCCGCCAGTTAATGGACAGTGCGCGAAAG ACATCAAGAAAGTGTCGCTGGAGCTGGCGGTAACGCGCCGTTTATCGTC TTTGACGATGCCGACCTCGACAAAGCCGTGGAAGGCGCGCGTGATGTGGC ATTCCGCAACGCCGGGCAAACCTCCGTCTGCGCCAACCGCCTGTATGTGC AGGACGCGTGTATGACCGTTTTGCCGAAAAATTGCAGCAGCAATGAGC AAACTCCACATCGGCGACGGCTGGATAACGGCGTCACCATCGGGCCGCT GATCGATGAAAAATCGGTATCAAAAGTGGAAGAGCATATTGCCGATGCGC Contig 32 (450 bp)

TGGCGGTGAACTATGTCGTGCGTGAAGAGCATTTGTGGTCGGTAGCGCGT

TATATGCGGGAAGTTTAGGCGAACTGGACAGCCTGGGTTTATCCGGTAGC
GAAATCCGCTTTCACGGTAAAACGCTGCTAGGCCTGGTGGAAAAAGCGCA
CACATTGCCGGAAGATGCCTTACCGCAGCCGATGCTTAACCTGATTGACA
GTGCCGGGTTATCGTAAAGCGTTTAAAGCGATTAAGTCGCTGGTGATATACTGAC
GTGAGCGAAACGCATAAGATCAGCGCCGAATTGCTGGCATCGCCTCGGCA
AATCAACCAACTGCTGAACTGGCACTGGAAACTGAACCGCAGAACAATT
TGCCGGAGCTGATTTCCGAGCTGCGTGTGAGCTGATGGCGGAAGCATT
ACACAATTTATTGCAGGAATATCCGCAGTAAAATCTTCCGAAGCCGGAC
GGGCGCGCTCAGCCCCCCACATCCGGCTTCGGCAAACTACAAATCCAACAC
Contig 36 (500 bp)

Contig 38 (450 bp)

AATGTACTGGCAAAAAGCCAATGGCGAAGCGTGGGGAACGTTACATGCTC
TGCTGGCGGATATTAATAGTCAGGGTCAGGTGCAGATGGCGATGAACCCC
GGCATCTATGATGAAAGCTATGCGCCGCTCCGTTTGTACATCGAAAAACGG
TCAGCAGAAGGTGGCGTTAAATCTCGCTTCAGGTGAAGGGAATTTCTTTA
TCCGTCCTGCCGGCGTGTTTTATCTCGCGGGAGATAAAGTCGGCATCGTT
CGTCTGGATGCCTTCAAAACCAGTAAAGAGATTCAGTTTGCGGTGCAGTC
AGGGCCAATGTTGATGGAAAACGGTGTAATTAATCCGCGTATTCATCCCA
ACGTCGCCTCAAGCAAAATTCGTAACGGTGTTGGGATTAATAAAACATGC
GAACGCCGTGTTTTTGTTGAGCCAGCAGCAACAAATTTTTATGATTTTG
Contig 40 (400 bp)

Contig 43 (450 bp)

Contig 45 (300 bp)
TGGGGACCCACTCAGCCCACTGAGTGACGGGCCCCCCTGTGGTCCCA
CCGCCAACCCACTCCAGCACCAGAGGGGCTGTGGCCACACCTTGTCCACA
GCCTGTCCCTGAGACCACGAGCCCCGGGCTCAGCCCCTCCTCACCCCT
GGACCGAGGAGAAGCCCCCACCTGGGCTCAGCTCTTGGAGCTAAACTTCC
AGGAAGGTTCTGGTGCCCTCGGGTCTTAGAGCATGGTGGGAGGGGGATG
CTGGTGGGGGGCCAAGCCCTCCCCACATTTCGCACTCGACCCGGTGGNG
Contig 46 (300 bp)

CCGGCTAGAAGCCACGAGAGCCCCCAGGCCCGACGTCTCTCCTGC
AGGGATTCGGCAGCCTGGGGCCACAGGGCCTGAGCAGACCTTGGGGTTC
CGGTGTGACTCCAGCCAGGGTCCCTACTGTTAGGCACCAGGGCAGAGTC
AGCCCTGGGACCATGGCCACAGCTGCTCCCGCCTGAGCCGGCCCCCGC
CCAGGCTGGGCCCCTCAGTGCCACAGCCAGCCAGCTGCTCTCCCAC
CTCCACCTTCTCCATCCAGGTCCTGCCCCACGGCCTTTGCTCAGGCCAG
Contiq 47 (500 bp)

GGGGTTGCCGCAGGCTGCTGTGTAGGTCGCAGACGCAGCTTGGATCTGGC GTGGCTGTGGCTGTGGCTGTGGCATAGGTCAGCCACTGCGACTC CGATTTGACCCCCAGCCCGGCAACTCCCACATGGCACAGGTGCAGCAGGG AAAATAAATAAATGAAATAAAAATAGGTGAAGACAGTGGATTTCATCTCT TGGGGTTGCGGTAAGCTCTACACAATAGGGAGTTTACCATTTTACCTGTT TCAAGTGGCACTGAGTCAGGCTCACAGTCCTGAGGGCCCACAGATGCCGTC TGCCTGGGAGATTGTTCCTCTCACCACACTGCCCCTCTGTCCCCACTAAA TACTCACTGCCCTCCCGTCCCAAGGGCCCCTGCCCCACCCTCTGCTTCC TGTCTCTGAACTTGCTGGCCACCAGCGACCGTCTGGTGACCTCACTCTTC GGCCCCATTTGTCGCACACCCCACCTGGCCTCTCCCCGGCATGGGCAGAN Contig 49 (600 bp)

GGCATATTTGGGGGGAGATCCCCACAAGGCATTTGGG GTTTGTGGTTTGGAATGCCCCCGGGCCCGATGGAGGGGGCCGGGGAAGAA TCTAAGCCTTACTTGGGGAGGGTTGGGCCCCGGGGCCCGGGAAAT GCCCCCAAGACAGAAGGTGTACAAAATTTCTCAAAAGGGTGACCCTTAAT GAMACGGGTCCCUGTTGGAAAGAGGTCACCAGGGTGGATTGGTGGCACCG CAGAATTTACGACATT'TTGGCTCTCTTCCAATGGCCGGACGCCTGGGGAT AGGUGCCCCGTGGACGGCGGGGGGTCTCGGGTGGGAUGGGCGGTCAGGGGT CGGTGACGCTTGGCCTCTCTGACCGCCTCCAGCTCCTTGGCCAGCGTGCG AGCGCGGCGGGCGCAGGGCGCGCGCAGGCCCTGCGCAGGCGTTGG GCGGACTGCTTCCAGGTGTCATAGCGGAAGAACTTGCCCACGGGGTATCT Contig 50 (179 bp)

ATCTTCATATTCATGCAGAAGACACTCTCCTGCCTTTCTATCTTGGGGAA AAGGACGATGTCACTTATGCAATAAAGCCCACTTGCTGGCCGGGGCTTGA CATTATTCCTTCCTGTCTGGCTCTGCACCGTATTGAAACTGAGTTAATGG GCAAATTTGATGAAGGTAAACTGCCCACC

Contig 51 (500 bp)

CTCGGGCTCCTTCCAGGGGGCCTTGGGCAGCCATAGAATGCTATGGAGCA AGAGAGTGCTATGGTUAGACGACTTTGGGGGAAGGTCTGGGAGAAGAGGG GTGACTGCCCACTGTGATAAAGAGTGGGCGCTTCCTTGAGATAACACGGT GGGCAGCCGAGCTGGACCTGTGCAGGTGGAGAAGGCCTCCTGCCGCGGCC AGTACGTGGCTCTGGGCTGCCGGACACGAGAAGCCCACCTCCACGGCTG CCTCCAGGCGGCCCTTCCTCTCTCACACCGCCGGGCCATGCCCAGGTGC AGGTGCCATCAGAGGGTGCTCAAGAGAAGCTCTGGGCTGGGGTTGTCCCA GGTCCCGGAAGCCCCGTGTCCCAGGGGCCACCTGAGGAAGCGTGGGCGCA CGCCCAAGGCGGAGGTGGTCAGAGGTCTTGGGAGGGAGGATGGCCGCGCA

Contig 52 (900 bp)

TGTGTTGCACCTGTTGCTGCCTGTCGACTCTAGAGGATCAATACTCCTTA CATAATTAAGGAGAACAAAATGGAACTTAAAAAATTGATGGGACATATTT CTATTATCCCCGATTACAGACAAGCCTGGAAAATGGAACATAAGTTATCG GATATTCTACTGTTGACTATTTGTGCCGTTATTTCTGGTGCAGAAGGCTG GGAAGATATAGAGGATTTTGGGGGAAACACATCCCGATTTTTTGAAGCAAT ATGGTGATTTTGAAAATGGTATTCCTGTTCACGACACCATTGCCAGAGTT GTATCCTGTATCAGTCCTGCAAAATTTCACGAGTGCTTTATTAACTGGAT GCGTGACTGCCATTCTTCAGATGATAAAGACGTCATTGCAATTGATGGAA AAACGCTCCGGCATTCTTATGATAAGAGTCGCCGCAGGGGAGCGATTCAT GTCATTAGTGCGTTCTCAACAATGCACAGTCTGGTCATCGGACAGATCAA GACGGATGAGAAATCTAATGAGATTACAGCTATCCCAGAACTTCTTAACA TGCTGGATATTAAAGGAAAAATCATCACAACTGATGCGATGGCTTGCCAG TGTAAAAGGAAACCAGGGGGGGGCTAAATAAAGCCTTTGAGGAAAAATTTC CGCTGAAAGAATTAAATAATCCAGCGCATGACAGTTACGCAATGAGTGAA AAGAGTCACGGCAGAGAAGAAATCCGTCTTCATATTGTTTGCGATGTCCC TGATGAACTTATTGATTTCACGTTTGAATAGAAAGGGCTGAAGAAATTAT Contig 53 (450 bp)

CCAGCCACCAGCTGGACCCTCCCGGAGAGGGGCTGCCTCCTCTTTCCCGC CCAGACGCCCCCAGCAATCTGTGGCCAAGAGGGGAGTGATACCGAAGATG GCCACATGGGGGCGCCAGCCCACAGGAACCCCAGGAAGGCGCTGGACCU TCAGGAGTCAGGGCTGCTGTGCACCCATGTGGCCTGGGGACTTTCCACAG CCTGGTGGAGATGGCCGGGGCACACCGCTGCCTCGGGGGAACGTGCACACG

GGTGGTACATGTGGCCGGAGCCCAGGGCACAGGGTGAGGGGAGAAGGGAG CATGCGGGTGCAGACTCGGAGCCCGCGCGTGAGGTGCTGGGTCCTCAGGA CACGCTCTGGGAGTGGAGGACCCCATCCACGCCCTCACCCAGTGTGTGC CCGCCTGCTCCCCCGGAAACCCTCACAGACACGAGGGCACACCCAGCCCC Contig 54 (1133 bp)

ATGGCGCTCATTAGAATTCGACCTCGGTACCTTGGGATCTTTTGACCCCT ACCTCACGCCATCTACAACATTTACCTCCGAATGAATGAGAGACACCAAA AGCAAATTCATAGAAGAGAAAAAAAGGTAACCTGGACTTTAAAAATGTAA ACTTCTGCTCTTTAAAAGGCAGTGCTAATGAAGTTCAAATACAAACCACA CACCATAAGAAAATACTTGCAAATCTTGTTCTGACAAAGACTAGTGTTCA GAACATACGACGATCAGGGAGAGAAACCAGCAATCCTATAAAACTGGA CAAAGAATTGGGGGGAAAAAAAACCCACTTGGCCAAGAAGTTGGTAAATA AGGCCATGAAAACATGCTCAACATCATGAGTCATTAGAAAAAATGCAAATT AAAATTATAATGAGATACTACTACACAGCTATTTGAATGGATAAAAAATG TTTTAAAAACTGATTATACCCAGGTTTGGCAAGAACATGAGAAACGAGAT AGAGCTGGGCACTTCCCTCAAAAGTTAAACATACATCCAGGACCTCACAC AGGCTTTCCACCACAGGTGTTTATTCCAGAGACATGAAAGCGCTCATCCA CACAAAGACTCGTAAATGAAGGTTTATAGCACCGTTTGTGGCCCGAACTG AGAAAACCCAAATGACCTTTAACCAGAGAATATCTAAACAAAATATCCAT TCACATTAATCACCCATAAGAAGGAACGGGCTATGGGGACGGGAACCGTA TTGAAGAGGGTCAAAATACATACGCAGCATCAAAGAAGCCTGCCCAAAGG ACACACACTGCAGGGTTCCATGGACTGAAACTCGAGAAGGTGAAAACTCG CCAGCAGTGACAGAGCAGGTCCGAGATCAACCTGATGTGGAGGAAAGT GAACCCTCGTGCGTTGTTGGCAGGACTATAAACTGGAGCACCCCCTACGG ACAACAGTAGCCCGGGCTCCTCTCCTCCATCTCCCTGGGGAGCCTGAGCC TTGAGACGCTGGGGCAAGTGCACGGCATGCTGCCTCACGTGGGGCCCCGG TGAAAACACGTGGCAGCTGGGGAAAGAATCGTA

Contig 55 (735 bp)

Contig 56 (500 bp)

GACCCCGTGCGTGGGCTCACAGAGTGTGTCCCTCTGTGACCGATCGTC GTGTCCCCGAGGCCCGTTCTGTGGCAGCTGCGTTATGACCGACTACCTTC GAATGCTCAGTGACTGCCGTGCATTGGACACGCAGTCCGCTACCCTTTTC Contig 58 (550 bp)

"GAGGAGCGCAGGCCCAGGCCTGAGTGTGCCCAGCTTACACCCCTGGCAG CTTCGTCCCTCCTGGCCCTAACCCCCATCCTACCCCAGCAGCAGGGGCTC CCCCGGTGGGGCCTGGTGAGCGTCTGACTGGGGTTTGGAGTCAGGTCTGC TOCAGGCTCAGCCCCATCCCCAAGGGTGCCCTGCAGCACTGCTGCCCAC CCCCTAGCGCCCCCAGACCTTCGCCCCTCCAGCCTGGATGTACCCACGGA CCCTGAAAACTGGGCTGAGCAGGTGCCCTGGCTGGAGTCCCCCTGACTT GGGGCTCGCCAGGCTGCCCTGGAGGGGCTGTGGGGGCACAGCCTGCCCCA TCCTGGCGGGCCACACCCTGCCCTGGGGTTTGGGGCCAAGCCCGGCACG CCCCATGTCAGGCGGGGGGGGAACCAGGTAATTACAGCCTGGCAGCCCGCT CCCCAGACCCCCAGCCCGGAGGGCCCCCACCCAGGCTGTGCCACCAAGA CCTGGCATCCAGGCCCCAAAGCAGGTCAAGGGCAGCTGCTACAGATTCTT TTAAGTTGAGACAGAATCGACACATGACAAGTTCCTGGTTTTAGGTACTT CGCTGCCGGGGCCGCCAGTCAGTTTAGTGACCCAGCACACCCCACACAGG TACAATTGCTCTTCTCAAAAGAGGCCCCTGAGAGAGCGCCTGTCTTGGCT CAGGGGTAATGAGCCCAATGGGTATCCATGAGGTTGCGGGTTCCATCCCC GGCCTCGCCGCGTTGGTTA

Contig 60 (500 bp)

TTTGAAAAATTTTCAGTCAGTGCAGAATTCGCATCTATTCCGCATTCAGG CTCTCCTGTTCTCACCTTGCCTTAGTGCGGATCTTCTATAACCACCACAG TGACGTTTTCAAGGTACTTTATTGAATAATAAGAAAAAAGTGCACACAAT CATGTAGTTAACTTTCTGTGCTCTTTTGCAGTTTTGAAGGGACCCTCTTTT

TTTCCTTTTTAGGGCTTCGCCGACGGAAGTTCCCGGGCTAGGGGTTGAGTCAGAGCTGCAGCTGCTGGCCTACAGCACAGCTCTTGGCGGCGATGGATCCCOntlg 63 (450 bp)

TCCTGGGCCACAGGCTGCAGCAGCTCACCTGGGGGCTGGGGTCTCGCTCT GCGGATGGACCCATGAAGGCCGGAGCCAGGTGGGGGCCGAGACGGCAGGG CAAAGGGTCTGCACACACAGCGTCCCCCCGACCCGGCTTCTCTGGGTTCT TGGGGGGTTGGCGAGGCTTCTCTCAGTCTTGGGTTTCCTGGGGAACTTTCA AGAACTGGGAAGTCTTCCAGAAAGTTGGGGTAGGGGAGGTACCCCCAAA GTGCTGCTCCTCTCCCCATCCCCACCCCGCTGTCCATCGGCGAGACCCC GGACCGCCGTCTCCCCGAGGTGTGGGGTCCCCCCTCTGCCGGCCAG GCTGGCAGGGGTGAGCCCCCTTCTCACTCGGACTCAGCCTGGC GAAGGCGGCCCCCAGGAGGTCTGCCTGCACTCGGACTCACCCTCCCC Conlig 64 (500 bp)

TGTGCATCCAACCCCAGTGGCCACGGGGGGTGACCCTCGGCCGGTCAGCC GCCCGGGTCTCCCACGGAACCGGGCCTTGGCCTGAGGCAGAAGGACCCAG GACTCCATCCCTGCCCGGACTCTGCCGGAGGGTGCGGTCTGCACAGAGA CCCTCTGGGGGTGAGGCCGGTCGGGGCTTGAGATGGGATGGTCAG GGCGCCCCCGCGGGCCTGCAGGAGGCTGGGTGAAGGAGGGGGCCCAGCT CAGACGCCCCCAAACCTAGCTTGGGAGAGCTGCAGCCCCGCCCCGTCAAT CGCGACAGCCTCCCACAGAAGGCATTCAAATGAGAGACAAATATTTGGG CTTGAAGACTATACCCAGCACGTCTCTTTGGGAGCCCAAGCTCCCCA GGCCCTCATTTGGGTATTAATTCGTTTTCGTTTAGAGATTTGCATGCTTA TCAATGGCCACTGGGCGGCCTGGATGCGGTCCUAGGCTTTTTATG Contig 55 (661 bp)

Contig 56 (500 bp)

TTTGTTGGATGAATGAAATCATGAGAAAGTGATTGGACCGCCCCGTTCUT
CCAGCTGCTGCCAGCTGCTTTGTAAAGATGACCTCTCACCTTCTCAGAG
GCCTGGCCGGCCGAGGTGGCACTCAGCTGAGATGCCATGCTTTTTGGC
ACGTGGGAGGCCCGAGGTGCACGTCAGGTGGAGTCCCTTTGTCTAATCAGG
GTCAGGGGGAGCAGCTGCAGGGCACATGTGGGGCCGGGGCCGATGTC
TGGGGAGGCCGGAGAGAGGGGTGTGCGGAGGCCGTTGTGGGGGTGCAGG
GGACAGACCCCAGCGAGACCCTCCCTGGCCAGGACCAGGACAGGTGATG
GGCGCCGCCTCCGGGGGGTGTAACAGAACCCTCTCAGAGGAGCCCTCC
CACGGTCTTCTGGACCATCAAGGGACCGGGGGCGCTGGGCTCAC
ACCCAGCTGGCCGGCCAGCCCGGGTGGGCCTGGGCCTTCAC
Contid 67 (550 bp)

TITGCATTCAGCTCGTACCCGGGATCCTTCCCGGGGGCTCTGGGGGTGGG

ATGTCAGGATAGTAACCTGGGGTGCTGCAGTGACAATGCCAGATCCTTAA CCACTGTGCCACAGGGAACTCCTTGACCTAGAATCCTATACCCACTGCA AATATATTCAAAAAAGGTAAAGTCCTGAGCAGAAAAUCAAAAATGGGAT AATTCATTTCTGGAAGACCTTCCTTGTTAAAGGAAGTTTTTTTGGACGTGA TGGAAACGGAGCAAATAAAGGTAAAAATAAAGTTCATCTCTTTCTCATTT TTTAATTGCTCCAAAAGATAGCTGACCTCTAAAGTAAAAAATAGTGGAAA TGTAGCATATGTCTCTAGCGTAATTTAAAGTATAACTTATAGCAATGATA GCCCAAATAAAGGAGGAATTGAGAATATACAGTTGCTGTGTTCCCCATTGT GCCTCAGCAGTAATGAACCTGGCTAATATCCATGAGGATGCAGGTTCAAT CCCTGGCCTCACTCGGGTTAAAGGATCCAGGGTTGCAGTGAGATGTG ACGTATGTCACAGACGTGGCTCGGATCTGGCATTTCTGTGACTGTGGCTG TGGTGTAGGCCAGCATCTGCACCTCCGATTTGACCCCTAGCCTGGGAACC AAAGAGAGAGAGATATACCATTGTAAATTTCCTCACATGACACAAAGAG CAATGTGATATTATTTGGTATATGGTGATTGATTCAAGATGTATATCATA NTATTGATTCAAGATGTATATATTCCTTTTCTAAAAAAGAGATTTATACA ATAAGGCAAGAGTGAAAATAAAGTGGAATGCTAAAGAATAGTTAATCCAA AAGAAGGCAGAAAATGGGGAAAAGACATATAACAGATGGAACAAATAAAA AAGAGCTAATGAGATTGTAAAATTTAATCCAAACATACAGATAATCCCAT. TAAATTTAAACACTCTCAAGACATTGATTAAAAGAAATTGTCAAATTGAA TAAACAAAGCAAGACCCAACTAGATGCAGACTATGAAAAACCCACTTCAT ATAAAGACATGGGTAGGTTTAGAGCAGAATGATGGGGAAACCATGTCACG CAAACATTTGTCAAAATAAAGCTGGTGTGGCTGTATTCATCTCAGACACA GCAGACTTCAGAACAAGAAACACTGCAAAGGATGAAAGAGATACTGCATA ATGATAAAGGGATCAATTTTCCAAGTGCAGGCTCCAAACAACAGAGGTTT Contig 71 (500 bp)

GACGTGCAGTAGCCATGACCTCTACGGCCCCCACTGACCAGCCCGTGTCC TTGTCCCGAGACCGACCCCTAAGCAATAGGATGCAGCAGAAGTGACAGAA CGGCCTCCGCGATGAGGTCGCAGAGGGCTCTGGCTCTGACTCAGGCCCCT CATCCCTCGCTCTCCTGGAGCAGGGCCAGGTAGGGGCCCCCCAGAGACGC CCTAGAGGAGGTGACGGCAGCCAGCCCCCAGGGAAGGCCTGGGGAC ACCAGGGAACAGACGCCACAGGCTCCTGGCACAGTCTCCCAGGAGCCCC CTGGTGGCACAGAAATCCTGACCGGCCCAGTGGAGGGGGGCTGGGGCGGGG CTCGGGGAGGAGGGACTGGGTGAGGCCGTCTGACTCCTGGCTGAGCGCCG CATACTTGCTGCCTGCCCACGATGCCGGGCCAGGCCTTCCGCACGGACCC AGGCTCACATTCGCCCTACATGCCACTGTGTGGGAGTTTGGGATGGTGTG CCCGCTGGGCCCGGGGTCAGGGCACGCTTCCCAGAGGAGCGGGTTCCAG AAGGCCCAGGTGGAGAGGCGATAGGAGGGCTCCAGGGGCTTCCCAGGCC CCCTTAGGCCAAGGCTGAGTTGTGACCGCAGGGAGAGGAGGAGAAGGAGCA CAGGGGCCCCTGGACGGCAGAGTCCCTGCTCCAGCTGCCGGCCCCGACCCC AGGTCCACCTTCATTTCACAGCCTGGCCCCGGCCGCTCTGACCGGCCCT GCCCATGCAGGTGTAGCGGGGCAGTGAGGGCCAGGCTCCGGCCGTCCCAA Contig 74 (450 bp)

CCTCCAGCTGGGCCCGCAGGGCACCGTGCCCCTCAGGGGACACCACGGGGGGCCACAGTGGCCTCTCCTGCTCCAGGCTCTGCTCCCGCCTGGGGCCCC CTGGGCCGCCCCATGGCCAGGGCAAACTCCCAGTGCGGCTGCCCGTC TGGGCAAAGAGGCCGCCAGGCCCCGCGTGGTCTTAGCAGGCACTGGCGGA TGCCGNTAACTAACCATTTCTTCCGCAGGAGT'CCGAATCTGCTCTGACCA CGGGCCCTAAAAATCGCTCCTGGCCCGCAGAGGATCCCCGAACAGCGGGG CTGCCTCCTGCTGCCGGGCCGGCACTCGGCAGGCACGTGCCCTC GTCGTCCCCAGTCTGTCAACCGTCCCGTCGTTACGATCCCCAGAGTCCCA CGCGCGGCAGCTCTTTCCACACCCCGCACGGCCCCGGAGCTGCCTGGGC ACCCAGATCGCCCTGACGCCTTTGCTCCTAATTCTGCTGAAATACACAT AACGTCTCCTTGAACGTTTGTCCATTTTCACGGGGACAATTCTGTGGCCG TAGGTACACTCCCCTTGGGGCGCAGCCATCGCACCATCCGCTTCCAGGAC GTCCCGTCGTCCCAGATGGACACTGTCCCCACTGATCCCTAATTCCCTGT CCCCCCAGCCCTGCCCTTCCTGTCTCTGTGGCCCTGGCGCCTCCAGGGA GCCCCTGTGCGTGGGATCACAAAACGTGTGTCCCTTTGCGTCCGGTGTGT GTCTCTGAGCATCCGGAGCTTGGGGTGCTTCCACGCTGCGCCTGTGTCAG GACGTECTTCCCTTTTGCGGCTGCGCGATGCTCCCCGTGGGGCTGCCCCA CACTGCGCGTGTTCGCTCATCCATCCACTAAGGCTGAGTTACTTTTGGCG GTTGTGAATACTGCTGTGTGAACACGGGCGTGCAAATACCTGCTGGAGGC CATGCTCTTAGGCCTCTCGGGGGGCACACCCAGAGCGGATATGCTCAATA AGGTAATTCTGTGTTTAGCTTTTTGGGGAACCATCAGGCTGGTCTCCAGA GTGACGGAGCATGCGTCGCATTCACAGGAATGGTGCTCGAGGCTTTGAGG TCTCCACCACTCGCTTCCTATTTTCTGTGCGTCACAGCCGTCGGAACGGC TGGGTGGTGCCTCTGTGTGGCTTCAATGTGCTTTTTCTTTTCCTGGCTAT GAGGTTGAGCGTTTTTTATGTACTTGCTGGCCATTCGCAGGGTTTTTTGGG GTTTCTTTTTTTTGCCTTTGGGGACGGCGCCCAGAGCGTATAGAAGT

TCCCTGGCTGGGGACTGAATCAGAGCTGCAGCTGCCAGGCCTAGCCCACAG CCGCAGCAACGCA

Contig 76 (500 bp)

TCATGCCATCGCCACCGCCCCCCCCGACGTTTCAAACACCAGAACCA CCCCTCGGGCGGCAGAGAGAGACGGAGAGAGACAGCCTGGTCCCAA CTCCGCGGTCCAAGTTTCACCCATCAGAGGCGCATTGTTTTCATCATCTG AAAAAAAATCTCTGTCTCTTAATAAAACACAAGAAAAAGTAGCCTTCGA AAGAAAGCACATGAATGATATGTGCTGGCGACAGTGCTGGCGGCCTCTGA GCCGTGGTGGGAGCTGGGAGCCAGCGGAGCCCCTGACCGATCACGTGACC CACGTCTCTCCTGCACAGCTGGCTGCACCTGCACGCGGTGACACAGGGAC CCAGCCTCCTGCCAGCAGGTCACCCCACCCGTCCGTCTCCTGTCGAAGG GGCAGCGTTGCCTTCTGAGGGTGGGCTCCTCTGAGGGGCGTCCTTTGGCC Contig 77 (626 bp)

TGCCGCCAGCCGCCCGGCCCCGCCCTCCAGACAGGACGCCCGGTCACC TTGCGGGGACAGCCAGCCTCGTGGCCTCGAGCAGAAGAAGTGAGAGTGGG GTGCACAGGGGCCCCCGGGGAAGGAGAGGGGACAGCGGGGGTGAGCGGG TCCTTAAACCGGGCCCAGCCTCTTGGGCCTCGACCCAAGGCTGTTTGGAA AATAGGTGGACCGTGGCCCTGACCCGAAGGCGGGGACCCCGAGTGCG GTCCCCAATGGATCAGCAGGCGCCTGGGACCCC GAGACACAGGTGGGANTGGGAGGAGGAGGAGGAAGGAAGGAGGAGAGGAG TGAGGACCAGCAGAAACCACGCCCTCCTCTCTCTCCCGTCCTCGCCCTCGC CTCCGACAGCTCCGACTCGGCTGCAAGGAAAAGGCCCCAGCCCAGCCCGG

Contig 78 (500 bp)

TACTCGGGTTTGTTACCACTGAGCCACAAAGGGGAGCTCCTAAAAATAATA ATTTTCTTAAAGCCAATGACATGGAGAGCAGTTAGGGTGGAGGCTGGTGG GTGGTGGGGCGGGGGGGCGCCCTGAAGGTCCTGAGTGGCACCCTTGGC GGGCAGGAAGAGCCAGTGGCTCCCAGTCCCCTGACCTTGCTGCCTT GAGCCTGGTTCTCCCCAAAATTCTGTCTGTGTCCCTTCACTTCACGGAAG CTTGGGGCCCGTTGCCAGGGAGACAGATGUGCTGGTGACACCCAAAATGA GECACCAGGAGGGGGGGCACTGACTTTAGCCAGCCGGTCACATCAAGAAGC AAACAGGCCCCCCGCTGCTGTAAAGGCAGCTTGGGGCTGGGGTCCGGGAG CACCCCTGGGCTGGGGAAAGGGGGTCCTCTCAGGCCCCCGGGGAGGATG Contag 79 (427 bp)

TCTATTCGCCGTGCCCGGAAGAGGCTAACCGTACATTGACCGGGCATCTG GCGATGTATCACTTCTCCAACCGAAACTTCCCGGCAAACTTGCTGCG TGAAAACGTTGCGGATAGCCGAATCTTCATTACCGGTAATACAGTCATTG ATGCACTGTTATGGGTGCGTGACCAGGTGATGAGCACCGACAAGCTGCGT TCAGAACTGGCGGCAAATTACCCGTTTATCGACCCCGATAAAAAGATGAT TCTGGTGACCGGTCACAGGCGTGAGAGTTTCGGTCGTGGCTTTGAAGAAA TCTGCCACGCGCTGGCAGACATCGCCACCACGCACCAGGACATCCAGATT GTCTATCCGGTGCATCTCAACCCGAACGTCAGAGAACCCGGTCAATCGCAT TCTGGGGCATGTGAAAAATGTCATTCT

Contig 80 (650 bp)

CGCGTTGCCGTGAGCTGTGGTGCGGGTCACAGATGGGGCTCAGATCCCGC GTGGCTGTGGCTCTGGCCTAGGCCGGTGGCTGCAGCTCCGATTCGACCCC AAAAAAAGGAAGAAAAGAAAGAAAGAAAAGAAAAGTCAAAAG GAGCTCCCCTGAGCGATGTCTGTCTACGAGCAGGTCCCTGGGAGCCTGAG GCAGGGTGAGCCCTGGACCCCTGAGGGCCACTCCAGACTCAGTGCTCTCAC GGTCAGAGGAGGGGCTTCAGGCTGCAGGGCCAGCGGCAGCTCTGGGCCCG GGGCGGGGGGAGATGGCCTGAGGGCCTTGCGGGGGCTGGAGGGTGGGGG GCTTCCTGGAGTGGGAAGACGGGAAGCCAGGTCACAGGAGGAGGAGCGAGG CCACATTCAACAGCCACCCGGCCTGTGGTCCTGGCAGGGTCCTGGCAGAA AAGCCCCAAGGGCCCCAGCCTGGCCCTCTGGGCCTAAAGAGCCAAGCCCC Contig 81 (550 bp)

TTAACCCACGGAGCAAGGCTGGGGATCGAACCTGTAACCTCGTGGCTCCT

CTGAGCCCAGCTATCTAGATTAGACCCCGGTCCGTCCCAAATTCTTCTCA AAGCTGTCCCGAGATGAGAGATGAGGTTTTCGTGTCCTCTCCTCG CTTCCCCTGGGATGTGCCCTAGGGTGGGAGAGGGTGTGTCCCAGGGCTCA GCAGGCGGTCCCATCTTCCCGAGACGGGAGAGATCCCCTCCTTCTCGGCG CCTGTCCCCACGGCCCCCACAGACACCCCCCCCCCGGCATGGCACCCAT GCACCTGCCATCGTGCCCAGTAGGGGATGGGTTTGGCGAGACTGGAGATG GCTGTAGCCACTGAGACATGCCCTGCCACGTAGCCTGACCCCCTGGGTGT GCTCTGTGAGATCTGGGGACCCCCAGCACCTAGGGATCATCTTTGCCA GCCTCCTGGGGAGCCTCTCAGAAATGGGGGCCCCCAGAAGGCTGGCAAAG GTGCGGGCTGGGGGGGGGGCTCCGGGGGTCGGAAGTGCTCCAGCAAGGT TTTGGACACAAAGTCAGGAGGAAGGAGTGACGAGAGACTTGCAGAATTA CAGGTAGAATCAGGAACCCACATCGACGCCAATTGATCTATCCCCCCCTT TTAATCCCTCCTTAGCTTTTTACGCGCTCAACACCAAATTAAACGTACTC AAGCCACCATCCGTCACCTTGGCGGCACCAGCCGCTGTCCTGCCCTCCGC CCATTTATCGCCCTTGAATTGATTTTTGTTTTGCTCTGTCCCTGTCGCTT CCAAAGATTTCAGGGGAATGAAACUGCTGCCGCC

Contig 84 (550 bp)

GAATCCCGGCATCGAGGCGGGAAGGGGGATGGAGGGATGGGGCCTACCCA CCCCTGCTCCCCACCCAGAATAGCTGGGCGCCCCCCATGGGAGGCCGCCC Contig 86 (913 bp)

CTGTTTTCACGTCTTCTGAGGACACACCUAGAAGAGGGGGCTGCAGGCGCC CATGGTGACTCCATGTGTTCACTGCTGAGGCCTCTGCAGACCGTCTCCCG CAGCAGCCGCACCCGTTTCCATGCCCACCACAGCGTGCGAGGCCGCACTG TCCCCACGGCTGTGCAACTGTTTTGAATCTGAGTTATATAAGCAACAGAC GCTCCTTCAAACACACTCACGTGCACACGTGCGCACAGGCGCACAGACAC ACACACGGAGTAATAGGCCTCCCCCCCCCCTCAGGCCCAGAGGGGGCCT GGGGCCCTGGAGCCTGTGCTTTAGGGCCTTTTAGGAAAGCTGGTGCCTCC CAGAGGGGCCCCGAGCGTTGGCTTCCCAAGTCCCCACCAACCCTCGA CAGACTCAAACGTTGGTTTCTTTCGTGCTTTTTGCCCAAGGGATGGGCCCG AGGTGGCCCTGCCTGAGGTTTCAGCCCAGGCCCCCAGGCACCCTTTCTCT CCCGGTCCCCGGCCACTTCATGGGACAGCGGGCCTTCCCCCACGTTGTCC CCTGGGTTGTCGTGCTTTCGTAATGAGACGGAGGCAGCTGCACCTGTCC TGGGGTGAATTCTCTTCTGCAGGAACTCGCTTCCCCGGCGCCTGGTCTGT CTSTTCCTCGGTTGTTGGAACCTCTCGTCACCAGAAAGGGTGGCTCTGAC GTCGCCCTTTCCCTCCGTGGCTTTTGCAGTCTGGGTCTTGTCGGGGAACC TGCCCCAAAGAGGGGAGTGACCCCCCACGAGGGAGACGTAGCTCCTGTGG CGACAGCACCGGGGGCCCCCAGATTCATGGGGTTCACGCTCACAGTCGCA TGACGCTGCCTTTGGACGAGGGCAGCTCAAGGGAAGCTTCTTTCCTGCCA CGAGCCACAGGCA

Contag 87 (650 bp)

TCCACACCTGTGGAGCCGCTGCTCGCTGATGCCCTCTCCCCAGCTGATG
GTCAGGTGCCCAGACTTGGGCTCAGTCCAAACAGGGCCCCACAGGTGCT
GCACCTGGGCAAGGGAGCCTTTGGGCCTGGGCTCAGGTGCTCGCCAGGGGCCCCACAGGTGCT
GCACCTGGGCAAGGGAGCCTGTGGCTCAGGGCTTCCCCAGGGCTCG
CTCGGGCCACCTGGAAATGAAGCCCCACCAACAACCACTGCTCAATAGGGTCCACGGGAG
CTTGGGGCTGCTAAGCCACCAAACAACTCAGTTAAGCGAGGGAG
CTTGGGGCTGCTAAGCTCCAAGCGGAAGCGGCCGCACTCAGCACTGCCT
CTCTGCCAGCCAGCCCCAGCTTGCTGACGTCCCACCAGCAGCAC
CCTGTCCCACAGATGCTGGCCCTTCAGTCTCTGCTCCCTGGAGGGGC
CGGCACTGTTGGGCACACAGCCCGCCTTTAAGGAAGGGAAAGG
CCCATCCTCAAAAAACCCGTGGCCAGGTGGCCATGATGGTCCTCCCAG
GCAGGTCCTCCTGGAACCCCTTCCTCCCTGGGCTCGCCCAGGACCGCC
CGGCACTGTTAAGTCTCTGCCCCGCATGTTCATTTCAAACTGGCTT
Contig 88 (700 bp)

GTCTCCGGCAGGGGTGGGCTCTGAACGTCCAGCTCCGCAGACAAATCAGA TTCCCCCGAGCCCTGAGAAAGCCCCCTCCCCAGCCCGTCTCCCCACCTG TCGGTGGACAGAGTGACCCCTGCTGACCCCTGCCCGGGCTCCCGCAGGA GATGTGAGAGAGTAAGAGGCGGTACAGGACGGCCGGGGCGGCCCGGGCGA GGTGCAGGTGTGGGTGTGAGGCTGGGCACAGCCTCCCT AGGGATCCACCCTCCAGCCACCTCCTCTCGGGCCAGCCCCCACCCCACCC CCGAGCTACAGATGCCTGCGCATTCGCCCCAAGTGTCCTGGACCCTGGAG CCAGGCAGCCCACCCGCTCAGCCTGGCCAGACCCAGCGTTGCCCTTCACG CCCTCCTCCCCCCCGGGGTCCTCGCGCTCGTCTCCTCAGGTTGGAAGC CCCTTCCCACCTGCCATCTTGCCTGCGCCCAGGATACACGGCTCAACTCA AGGCCTCACTCCTCGCCCTCTCCAAGGCTCTGTCCAGGCCCCTCTCTGACCCTGGCACCACCTGCCGCCTCCTGGCAGCCCCAGCAAACCCCCTGCCACAG TCCACGACAGTCCTCTTCTGGCTCTGCCCCCAGGATGCTTCTAGAACTGG GGGGGGGTCCTTCCAGCCCACGCAGCATCCACTGGGCCCTGGGCTCCCT CCCCAGGTGCCCCTCAGAGCTTGCAGCTGGTGCAGACGGCTCTGCTCCGA ACCCATGCTCCCTGCGCCCTTGGACCTCGTGAGATGTTCCAGGTCATTTG GCTGCACCCAAAAGAGTGGCCCCTCAGGGTCCCCCCTGCGCCCCCTCCATC

Contig 90 (350 bp)

Contig 91 (1464 bp)

TCCAGGACCTGATGCAGCAGCCACGTCGCGAGGCCCCTCCCACGAGGCCC CTTGTTGACCAGCGCTAGGGAAGGGGACÇAGGGAGATGCTGAGAACGGGG CCTTCCGAGGGGGCAGGTGGGACTGACTGTGACCCAACACTCCCCACCCC AAGCTGCCTCTGGGCGGGGTGCGGGGCTGCCCAGCAGGGTCCCATCCAG CTTCTCCCTGGGGAGACTCACAGTTCTGGGAGAAGGGTCCTGACTGCACC TGACTGGTCTCCGCATTTGCCCAGGCTGGGCATCTGCCCAGAGGATACGT CCAAAGGCAGGCAAAGCCGGGCCCGTCCCCGGAGCTCCCCACAGGCGC TGAGGGCTGGGTCTCGGGGGGGGGGGGGGGGGGGGGCTCAGAAGGTG CAGCGGGGTGGAGCGAGGCTGAGCCAAGGTGCACGCGAGGGCCACAGAAG GCCGAGGCGGGCAGGAGAGAGCCCCAGCCTGGAUGGGGGTGGCTGCC CTGGGCAGGTCTGGGGGCTCAAGAAGAAGAGTGTGTGTGCAGGGGGCTG TCCAAGCTGCCCGGGAGGCTGCCTGCCACCTCCAGGGAGCAAAGCAGGGAGCTGCCAGGACCACGCGTGGCCCAG GCCTCAACGCTCCTCCCACAGCCCAGGAGACCCAGGGCACCCGGTCCATT TACCGCGGGCTCCGGTTCGTTTGCCTGCGCCCTGGGATGGACTGTGGGG GCGGGGCGCTCTCTGGGGAGGAGGAGGTGTCTGAGGCTGGACACCTTGA AGGCAGGTGAGAGTGACAGGTCCGTGCGCAGCAGCCTTCGGCTCTGGATT CTGGCCCTGAGCGAGGGGCTGGCTGGAAACTGGGCCGGGGCTGCCCCAGG AGAGTGTGCAGGGAGAGGGGAGGAGGTGCCGGGGTG GTGCCCTGGAGTGCGGCTGAGCGGGAAGTGGGTGTTGGCGTCTGGAGACG GGGGGTCGTGGGCTTGGGATGGTGACAAGACCCCCCAGGTGGAGGCGGCC AGTCAGGAGGGACCAGCAGAGCCCTGGGCTCAGTGTCACCGGTCCTGGCA CCTCGCCGACGGATGTCCTGGCCGTGCAGTGGTTGTCCGCTCACCCTGAG CCCTGAGAACCATGCAGGATGCTGGTGTCACAGCAGGAGAGGGCCAGGGC CTGGGGAGGACTTTACTGGAAGGCCTTCTCCTTCCGTTTGCAGCAGGCG GGAATGACTGGGGG

Contig 92 (694 bp)

TGGAGCCAGGGCACGCAGAGCGGTCCCGAGGCCGTGCTGACCCGGGGGATGGGCCGGACCTGGGGGTGGGCTGTGAGCCCAGGCATAGGGACCCCG

Contig 93 (900 bp)

CCAGCCCCATCCCCCGGCTGGTCCCCCCACACACAGAGCCCCCGTTTCCC AGGGGACAGCACAGCCTGCCCCAGGTCTTACATAAAGTCACCTTCTCAG AGCTCCTGTCGCGGCTCACGGGAATGAATCTGACCAGCATCCATGAGGAC ACAGGTTTGATCCCAGGCCCCGCTCAGCAGGTTAAGGATCTGGCGTTGCC GTGAGCTGTGGTGGAGGTCGCAAGACGTGGCTCAGATCTGGTGTGGCTGT GACTGAGGTGGCGGCCAGCAGCTGCAGCTCTGATTGGACCCCTAGCCTGG TAAATAAAAAGAAGTAAACACACCTTCTCTAGCCATAACCACCTGCCTAGG GGCGGAGGGCCAGGAAGCGGCACCCCCCCCCCCCAGGCTGCCCGTGCGCCC CCCACATGGAGGGGCTGGGCTGCGCAGTAACTGCTTTAACTGACGGGAGC TTCGACCAGCAATTCACCAGCGGGCATGCAGCCGGGAAGGGAAGTTATTC GTGTGTAGCTATTAGGCGCCGGAGTGAGGGTGTGCCTCGCCCTGGGCCCA TGTCTCTGAAATCCGGGGAATCCCCACTGCAGGCATGTTCAAAGGGTCAA GACCGGGGCTCTCCCTGAGAAGGACTGGCGAAGGCCAACTACAAAAGCGC ACCCCTCTGTGCAAACCCCCAACCAATGGAACAAAACTCCAGAGGGGCCA Centig 94 (550 bp)

GTTTGCTCTCAGCAGGCAAGGCCCTCCGAGGCCTTAATAGCCCATAATGA CACCGCCCGCTCCTGGCATGGGGCCCCCCCCTGGCATGGGGCAGGGCAGGG CAGAGCAAGCATGCAGCTTCTACCTTCTTCCTGACCTCGTGGCCCCT TCCGAGGCCTCAGGGGGTCCCCCGAGTGGGACCCCAGCCCTGGCTCTCCT CTCCAGAGCCAGGCCCAAGGCTGGGAGTGGCCCAGAGATGAGGGTGCCCG AGCAGGGCACTGCCTTGGCGTCCCCATCCCTGGCGCCTCAGGGCCGTACT GTCCAAAACCAAAAGAAGCAGTCAGCAAAACTTUTCCCAGCAAGCTGGG GTCAAAGGTCGCTTCCGAGGCGTGATCAGGGTGGGCTTTGCTACTGTCAC CGTGTGCCCTGGGAGAGGGACAGGGACACACACACCTCCGAGAACC TGGGGCTTCCAGGGCGTCAGGCTGCCTGGGCCATCCCGGGCCCCTGTGGT CCCAGGATCTGCCGGGACCGTGAGGCCTGCGTCCCACCCTCTGCCTGGGA CAGGCCCCACAGAGCTCACAGCCAGGGGACCGGGGACAGGGCCCCGCCTG GGCCACCTGCCTCCAGCCTCACCCAGCCTGGGCCCCAGGCCTGTGCCTGC GACACCCTGAGTCTCAGGACGGGCGCGGGACAAAGCCGCCCGGCCCCTCC CCCGGCTGGGAGAGACCCGCGTGGCCCTGACGTGTGGGCCTGTCAGAGC GGAGGCCGGCGGAGGGGATCCACGAGCCGAGGCCGGAGCTGGCCACCC CACCGGTCGATTCCAGGCACTCAGGGATAATTCGGTGTTTAGAAGTCAGG TTAACAGGTGCCCGAACACGCAGGTCTGGGGAGATGCTGAGGTCGCCAAG

Contig 97 (1350 bp)

CCCCCCTTATTTTTAAATTTCCGAAAACAAAAACCACACCTCTCCCGTCC CCGAAATTATTTTGGTATAGTCTTATTCAAAGAAGTCCTGCCACTGAAGC CCACTTGTCCTGTCCCGGGCTGCTTTGGCCAAGGGCCCTGACGGGCCCAG GGTGGCTCATTCCCGCATCCCGCAGAGGCCGCCTTCACATCCCATGCGG GAGCCTGGCTTCCGGCACCCGGCTGTGCCCTCGCTGTGGCCATGGACTGC TTTCGCAGAAGCATAGGGGCCACAACATGGGACAGCCTCGCTCTGCTCGC TGTGGTTCCGCTGAACCTCTCAGCTGGACATCTGGGCAGCAGCACCCCA GCTTTGCTTCAGGCTCTGCTTCCAGGCTGGGCCCTCCTCGGCCCTGCCCG CTGGGTGCCAAGCAGGGCTGGTCCCGGCTGTGCCCCCGGGTCTATAGAAGC CTCTGCAGGCTTCCTACAGCCAGGCTGCGATTCGGCGGCTGCCCGGGAC TGAGGCCCCCTCTGAUTCTGACCCCCCATCCTTCCCTCCCACACAGCCC CCCGCCCCCGCTTCTGCTTCAGTGAGGCCCCACCCTGCCTCACTCGCTGA CATTTCCAGAACAGGGGGTTCCAGGAAGCCCTGAGCCTGCAGGGGACTCA GTGACCAGCCGCATCTGAATTTTCCCTCCTTCTGATCTCTGGAGACACGT CTGGCTCAGCCTGGCTCGAGTGCCCTGAGCTGGGGACCAGGACAGACCTG CAGATGGAGGTCTGAGCCTGGGCAGGGCAGGGCCCAAGGCTCAGGGAGAA ATTGCAGGTGTGAGATCAATGACCGGAGCCTGGATGGGGCCGCCCTGGCC AGGGCAGCTTTCTCCCTGCAGCTCCCTGCCACTGTCCCCCCAACTCTGG GCTCCTGCTCTGGACCCAGTTGTGTGTTCCCCTCCTCCCAGCCGACCCAC CCTCCCCATTCTGCCCCCCCAATCCAACACCCTATCGTGGGAACCAGT GGAGCTGAAAGAAGGACCCCCCAAGGGCCCCCCAGCCGCTGTAATCCTTG GGGGCCTCTGCCCAGGTGCCAGGTCTCGGGCAGGAGGGGCCGCGGGCACA GCCGTGGCAGATGCGCCCCCAACCCTGGGCTCGGAGGAGCCCCGCCCCC ACTGACATTTCCAGGCCGCCCCCCTGCAGACCCGGCTGGCCGTGATATTTA GACAGGGCTTATTTGCCGTGACTGGTTTTTGATGACTTTGGGGCCCAGGA TGAGCTCAGCCGAGCCCCCCTTGGCCCACCTTGGTCTCAGCTTGGGTTTG ATAATATAACGCGTTCAACTGAACCGCTGACGCCTGCGTGGGCCGAGGCC Contig 98 (1354 bp)

GCTTGCAGTAGTTCATCAGATTGGACGACTCATAAATGTCAAGACATCTA CAAGAAGCTGACCCAAAAACTCACGTGGAAATGCACGTCAACTGGGAEAG TTGAAACAATTTCTAAAAAGAAGAAGGACGTCCTGGGAGGACTCTTCGCG CTCTTTGGTTTCGCTTCACTTTATATTATTAGTTACTGATTTTCCTAAAA GGTTGGGACAGAATAGAAAGCCCAGAAACGCACCCCCGCAAATGTGGTCA ATTGAGTTTGGGCAAGGATGTGAAAGCGGTTCAGTGGAGAAGAGTCTTTT CAAGAAATCTCTGGTCCTGGATCCACTGCTCATCCAGGCCCAAGAGTGAA CTTGGCGCACATTTCTCACAGTGTATACAAAAACTGACTCAAAATAATTC ACATACCGTCGTGTAGCGTATGAAGCCATGAAACATCCAGAAGAAAATCT CGGTAACCTCAGGGCATCTGGGGCCTCCACCCTCAGCACCACTGGCCTTG GGGCCAGATACTTACGTGTTCTCCTGTGCACTGTGGGACGTGCAGCCAAA CCCCAACAAGGTGACCATCAGAAATGTCTCCAGACGTCGCCAAATAACTG CCAGAGAGCACAGGAGCCCCTCACTGAGAACCACAGGGTGGGGCAGAGAG ATCTCAGACATGACACGATTAGGGGAAAACAATCTGACACACTGGCTTTG TTAAATTTAAAACTTTTCCCCTGTAAAAGGCAATGGTAAGACATTAAGAG GCGAAGTGGCAGACTGGGAGAAAATATTTGCAAATCATGTATCAGATACG

Contig 99 (1000 bp)

CGTTCTCAGGCGCACGGGGCAGAGGCTGAGGGTCCGAGGGGCTTTGGGTG CTGGAAAGCCTGAGTTTGAATCCCAGCTCGGTTTCTTAAAGCTGTGTCTC CACGGCCAAGGAATGGGGCCTCTCTGGGAAAGGTCTGGGGTGAGGCTGGC GGGACCTGCCAGCCCCGGAGGGCATCTGACCAGACAGCTTCTCAAGCTCA CAGGGCTTCATGGCAGGATGGGGAAGGCTGTGGTGGGGAGTGGGGAGCAC TCGACACCCTGTCCAGGCCTCTTCAGTCACGGTGGCCTCTCAAAAGGGGT TOTOTGTGTCCAATGAGCAAGTCTTTGTCCGGGGCAGGATTACTAAGTCC AAGGGTGTCTGCCCCTCCGTGGGGCACAGAGCAGCGGCCCCAGATCACGT GGCTGTAACTGCCAGGTTGCAAAGCCTGCCACCATGTCCCACTGGGTTCT CCAGTTACCTTGGGAGGTGCAGGGTGGGGTGATGGGGAAACTGAGGCAGA GAGCTGGCAAAAGAGTGCCGGCAGGGACTGCGGCCGCCAGACCCAGCTAA CCGACCCTCACACGGAGCTGCTTCTACTTTGCAGCCTGGACGTGGGAAAA GGTTACCCCACAGCAGCGTGTGCAGGCACGCTGGTATGTCTGTGTACTTA TGCATATGTTCTACGTGCATGCACGTGAGTGTGCTUTGTGCATTGTGCCT GTGTGTGTGCATGTGTGTGTGCACTCATGTGTCTATACGTCTGTGTAG TGAATGCTTGTGCATGTGTATTTGCATGTGTATGTTTGTACGTGTGCAGT GAATGCATGTGTGCGGTGTGCGCATGTGTCTG TTTATACCTGTGTGTGTGATGCATGTGTGTGTGTTTACATGTGC ACGTGAGAATGTGCACTCGTGCATGTTTGCATGTGAGTTTCATGTACACA TGCTTTTAACGTGTGCACGTGTGCACATGTGTTTCTGTGTCCCTTGCACG Contig 100 (1500 bp)

CGTATAAATATATAATATAGAATAGAATAGATTGATAATATAGATAAAC TAAACCCATTATCAATACCGGGTGGCCCCAGCAAAGGATACTAGCCAGTT TATCAAGGTGCTAAGTCAGCACATAGAATGGCCACAAACGAAAACCTGTA CTGCCTATGTCCACTCTAATGGAGTATGCCACTGACATCAGTGGTAGGTG AGCTGAGTCCATCTGGGCTCCCAGTTCGGGCCCCGGCTTGTCCCCCAACGG AGGTTCCTTCCAGGGTTCCCCAAACCCAACCGGGCCCCCAGGTCTCCCTG TCTTGACTCGTTTCTGGAGTCTTCTGGGGCTCTGCAGTCCTCCCTTGTTG GGGCTTCTGTCCCCCTGCCCCTGGCCTTGCGGGCTCGGCCCTGCCCTGGG AGGCTGGGCCGGGCCAGGAGGGAATGCGCCTGACTCTGCTCCAGATGGAC AGGTCGGGACATGCAGTGGCCTCGCCTTGGGCTGCTGAGCCAAGAGCAGG CAGCCGCCAGCATCTGTCAGGGCGCTGCAGGCGCGGGGAATGACCTCGA CTTCTGCTTGGCACCCAGCTCTGGAACAGCCCCCTGCGGAGCCTCCGCCCAGAGCTGGGCCAGAGGGTCCCCTGTGCCGGGGACCCCAGCAGGGCCCCTC CCTGACTCTCCAACCCACCTGCCTGGGAGGAGTGGCCCCCTGGCCTCCGT GGATCTCTGGGTCGGGGCTCAGCCGGCTTGACAGCCTGGGAACAGCCAAT GCACATCCCCAGGCCTGGCCACACCCTTCCACCGGGAGCGCGGGATCTG CATTTCGCCAGGCTCTGCGGGCAGCTCTGAGAGCCCCGGGTCTCGGAGCC CAGCCGTGGCCGTTGTACGCCCTGGGGGCTGTGGACAGCGTGTCCTCATT GCCCCTCCGAGGTCCGGCCCAGGTCCCCTCCCACCTGCTCGCCCAGAGCC CCGGTTTCAGGACCTTTGCACGTGCCGCTTCCTCTGCAGAGAAATGCCTG GAGCAGATGTTTGTCCGCACGGCTGCTCCGCGAGGCCTACCGAGAGCCCC TCACCTAAACGGCCGGGCCTCAGCAGCCCGGGGCCCTGTCCCCACCGCCC AGGTGGTGGGTTCTCCTGTGCCAGTGTGGGCATCTCTGTAAGATACCTGT TTATCTGCTCATCGTCTGGTCTCCCCCAGAAGGTAGAGCAGGCCCGGCA CAGCCGTCCTCGGGGTGGCCACTCGCCCTTGGGGCTCAGCCTCCATGCAC GGAGGGACGCCTGGTGACACGAGAGCCCCGTGTGAGTGTGCCGGGCCGCC AGCCTGCCTTAGGTCACAGCCAAAGCCGGCATTAACCACCAGGCCCTCGA

Contig 101 (600 bp)

AGTATATCGGGTGAGACTGGGGGACCGGTCTGCCGGGAAGCCCCACCATAA AGGCCACGTTGGGGCCACAGTCCGGGCCACGTGAGTGTGGGCGGGTCCGCG GGTCTGCTCTTGGAACACCAGGATCTCTAAGAGGTACCAGCCGAGGCCAA CTTCACGTGAGCAAGTGAGCAAATGACTGAATGAGAGCCTGAGCGAATGA GTGAGGGGTGAGTCCGTCCACCACGCAGCCTAGGCTCAGCCAACCGCTGT CCCCGCSTCTCCACTGGTGACCAGAACGAAAGAGTGGGGAAAGAGTGGT TGTCTCCCACACCCAGTCCCCAACCCCCTGGACGCCCCACCCCTCCAG GGGTGCCGGGCCTGTGGGCCCCAGTCTGGAGGCTCTGGCACCTTC CTCATCEGTTCTCCCAGCACCCCAGGTTCGTGCTGAGCCCTCCTGGCCCA CAGGCCTCGGGGACAAAGAGGGCCACCTGGAGGCTCAGGGAGCCTCACCT GCCTCGTGGTCCTGGCGGAGGCGGGTCTGGACATGTGATAGACCGGCCTG GGCTCAGCAGCTCCTGCTGGAAGATGTCAGGGACAGCCTGGGCCACTCTC CCACCAGGAGAACTTATTCCTCGGTGGGGTCCCCCCGGGGAAGGGATGGG ATCCCAGCGGGGACCCCAGAGCGTCCAGCACACGGACCTGTCCCTCCAGC CCCTGCCCCACACGGATGCTCACAGCTCAGCCTCGAACACGCACCTGTTG GACTTTGCCTCCTGAGGCTGTCTTCTCAGCCGACGCGGGCCTCCGCTGCA TGGTCTGGAAGCCCAGTGGGACTCGCTGGTGACAGGGAACAGGGGCTCTT GGAGTGGGGTGCCGGGGGAGCCCCGAGGGACCTGCTTGGGCCTTTGATGG CTGAGTGGGCTGAAGTCAGGCAGGCTCCCCAGGCCTCCCTGACCCCCCC CACCTCAAAAAATCCAGAGCATCCTTTCCTTTGGGTCTGGTGAGGCTCTC TGAGGTCAGACCCTGCGTGGCTGGGCCAGTGGGGGCTGGAGCAGGAAGAAA GCAGGACAGCCCCCGCCCCTGGCCCAGGACCCCCAAACCCAGCAGGAGAC ACCTGAAACGGGATGGAACCATCCTGAAAAGAGCCACCTCCTCCTTA TGCATCAGCTGCCGGGGTCTGGGGGCCCGCCCCAGGCCCCAGATGTCCGG GCTGCTCCCGTCTCACATCCAGGGGTTTCTGGGCCCAGGACTCTGTCCCC TGGCGCCGGGGACAAAGCCGGCTGGGGTCTCAGGTTTGGGTTCAGAGCA AACGTTGATCTGACCTGGTTCTGAGATGCTCGGCCCGATGCTGCGTTGTC CCGGCCAGCCCCACGGAGGGACGCCAGGGTCTGGGGGGCC CCTGCCCGCACCAGAACGTCTGGCTCAGCTTTTTGTCCTCGTGACCCATC ACTAAGGCCCACCCTCTGACCCGGAGCCCTGTCTCCGAGGTGGGAATTGG GGGCTGTCCCTGGCGTCATAGGACCTGGTTGGGGGCCATCCAGGGCTGTGT CATGCCCCTCCCCAGAAGACTCTGGGGGGCTGCGGGAGGGTTTCCCCAGCT TCGGGCCAGCCTGGGGAGGCCGGAAGGCGCTGCAGGCCTTGCCTGTCCCA **GGCCGTCTGGAAGCACT**

Contig 103 (650 bp)

GTTGAGGATTCCTCGGCAATTTCCTCGTCACTGGCGCTCCAATCGCCTCG
ATGGGCTTCTCCTCAGATACAGCTGCAGATCCTGGGCGGCACACCGTT
GAGCGTCACCTCGTACTGCAGATTCTGTTGTCAATGGACATCCAGG
CCATGCCGACGGCATCTGGATTCTGTGCATCCGTCTGCCTCCTGTCGCTTC
AGCAGAATGGGTTCCGCCGAGTCCCGAGCATCGGCCACTGGACGGGGCAC
TAGGCGGCCACGGATCAGGCTCCTCATGCTCGGTGGCCACATTAACGC
CCAGTTCGCCGGCATACAGCGACTCGAGACCTTGGGACCCAACTTCTCC
ACACTACCAATGGCTGGTTGAAGTTGAAGCTCGAGTCAGATCCTCCAG
CTTGGCCTTCCCCTGCTCCATGTAAACTGATGTTTGGCCTAT
CCCGGGTGTTCACGTCCCGTTTCGATGTTGAGCCAGAATCCATCG
GTGTTCAAGTAGACCCACGCCAAACCGCTGCTCTTGGTCGAGGATCCATCG
GTGTTCAAGTAGACCCACGCCCAAACCGCTGCTCTTTGGTCGAGGATTCGGC

ACTGTGCGGCGCCAGCAGGGTCTGGAAGATTTCGCAGCTGGCTCGGGTCA CGATGTGTCCCTGGATGCGCAGATGTGGGTACTTCTTGGACTCCACGGTC Contig 104 (1630 bp) GGTGTTGTCACTGCTGTGGCTCAGACCCCTGCTGTGGCACAGGGTCCATC CTTAGCCCAGAAACTTGCACATGCCACAGGTGCAGCCAAAAGAAAATTCT TACTAATAAGTTGTTCATTTGCCTTTACGTAGAGTGGCATCAAACAGCAA ATTTAAAACACCATCTATCAATACATAGACCGCGGTCAAAGGGAAAGAAC TTTCTATTTCAGCACCTTTAACATGCCTTTGCCCGAATTTCGGACCAGGG TGCTGTGTTTTCATCTCTCCCTGCAGGTGGTCCCCAGATGACCAGGCCGG TCCTGGGCGGAGGAGCCGGACTGTGGATCCAGTTGCTTCCCAAGACAGG CTGACAGGAGGAGCAGGGCCACCCCAACCGAAACCAAAGCCAGAAC GAGCAGAAAGATGCCGTCTTCCAAGTGGGGGCTGGGAGCTTCUTCCCATC CTCCGGAGCCGTGAGCTGCCCTGGAGCTGGCAGGAGCCACAGAGGACCC GGCTTTGACCGCCCCTCTGGGACCCACAATCAGGACCCTGACTCAGATGC TGAGGGGCCTGGACACACCCCAGGACCCTGCTGCTTCCCCAGAACCGCT CCGTGGGGCAGGCTTTCCCTTGGGCACCGATGCACCTTGAGGGCAGAGAC GGGGCCCAATAAACGTTTCCAAACCAGTGGGTGAGGGACCCGACCGGCCC GACACCGCAGCCCGGATGCAGGGACTCCGTGCTTCGCCCAGCCTCCCTTG TGGGGACATCCGTTCTCTGATTGGGTGAGTTTCAGCCACAGAGATATTCC CAGGACTACAAAGCTGGGTCCCTTGGGGCACCTGCTGTCACAAAAAGACA AGGCCCTGACCCCAGTAGCCAAGTTCCCCCAGGGGCTCCCCAGGGTCTG GAČTCTCTGTAAACATCCCCCGGCCCCACCCAGCTTTACCCCAAGGCCGA AAGCACCAGUCCCCCTGCACCACAGATGAGGCCCCCATCGCTCCCCGACC TAACTTCTGTCTGCAGTTGGCTTTCAGCCTCGGGTGGGGGCAAGCCCTGC ATCTCAGGCTCCCGGGAGAACTTGCTGCCTCCACAGCAGAGCCAGGGGGCC TGCTGACCACCTGGGCCGGGTCGGATCTGGTCTAGAATGCTGCTAAGGTG TTGCCAGGACTCAGGAATGAAGCCATCCCAGGTTTTGAATCCCCGGTCCC ACCACCTTCCACCTCTGACCTCAGGCACCTCGGCTTTCAGAGCTGCCCTT TCTGACTCTGGGACACGGGGCTGTGAGGCGCTCTCGGTGTGACAGCTG GCCCTTTCCTC'/CTCAAAAACGCCCGCCCGAGTGACCTCACGGGAGGCAG

Contig 105 (1820 bp) AGTGAGCCCTGCAGGACAGTCTGCTGAGGGGTGTCTGGGCTCCTCAGAGG CTCATGGCCACCGGCACTGGGAGGATGGCAGGTGGACCCCTGCATCCAGG TCCCAGGTCCCAGGTCCCAGACCCCGGACAGGCTTTCTATCTGCAGGAC GGGGGCTCCTGGGGCAGCAGGGATGTGGCTGTGAGGCCTCGTCAGTCTCC CACACACACACGCACGCACGCACACACAGAGGCGTGACCAGGGCTGCA GACAGGGCCATGGGAGGACTGCCCGGCAGTGCACCCAGATGGCCACACGG TGGGGCCCTCGTCCCACTTTTGCTGCTGATGCTTCCGCCCAGCCTGCTGG CAGCAAGCACTAGCTTCCCAGGGCTCTGACCAGAGAGGGATGGGAGGGGT CATGGGTCAACAGGCGCCAGGGAATGGGGAATAGGATCTGAGGGGCGGGG GCAAGGGGCCCAGGCGAGGCTGCACTGCCAGAGCTCCCTGCACCTGCAG GACCAGCCACAGGCCAACAGCTGCAGGCAGAGCAGGGCTGCTCCTGTCCC CAGAAGCTGGCACAGCACATGGGGTCTGACAGCCCCACCCCGGGCCTCCC ACAGAGGGGCGGGTCCCCCAAACTCCTCCCCCGTCCCACCCCCACAGCTCA GCACGCACACATGAATGCACCTGCAAGCACACACTCACACGTAAGCAG CACACACGCACACACTCAAACACGTACATGCAAGCACATGCTGGTCCT TTGTCCCCGTCGAGGGGAGGATGGAGGCCCAGCCCGTGGGGAGGGCATGT GGAGTGTTGGGGGGCTGGCTCCAACGCCCTCGCTCAACAGGCACCAACGC TGGACTGAGATAAGCCGGGGCGCTGGCTCCCTTGGGGCCGCTCAGCAGGT TTGACGCCCACCACAGGTGGCACTGCCTCTTTCAGAAGACGGATGTGGCC ATGCCACCCTCACAGCCTCACCAGTCCCCCCTCAGCTTTAGTGCTGTCCC TGTCACTGTACCCGGGGCCTTCCTTCTTCCAGGGCCAAAAGCGAGTTCAG GGGACAGTGGCGCCCCATAATTACTCACCCAGGGTGCTGTCCTCTGTGG TGGCCTTGAGGCCAAGGTGCTCCCATGGGGGCCCACAGGGCTGGCAGGGT

GGCCAGGAACCCCAAACCAGAATCA

Contig 106 (1500 bp)

TGCCGAATAGAGGTGGAAACCAAGACCCGAAAAAATGTCCACATTTTTCA ATTATTAGAAATTTAGAAAATATTTTACAGGAGTTAAAAGGTATTCCAT TCTGGGGGGGGGGGCATGCCCACGGCATGCAGGCATTCCCCGACCAGC CACTGAACTCGAGCCACGGCAGTCACCATGCTGGATCCTTAACCTGCTGA GCCCCTGGGCAACTCCAGACACTCCATATTCATGTAAACTATTTTTTAAC CAAAAAAATGACAAAGCTTTTCAAAACAAAACACATTTCATGGGAAGAGT GGCATTGCTTCACGCCTGGATGGTCGCTGCGGCTTGCCGGACGACGACGAGGG CCCCGCGGGAGCGCCTCCGCACGCCGCATCAGGACGTGGTGTCCAGGGA AGCGGGGTCACTTCACGGCCTCTCGGGTGCGCGTGGGTTTCCTTTTCGGC ACCACACCCGGACTCAGCACTTGGGGGTTCTTAAACGTGAGAGGCACTGC GGGGCTCGAAGCCACATCACTGACCTCCTCAGACTCTGTTATGTGAAAAC CCATCCGTCCACGAGACCAAAGAGACAGACGCAAACGCAAGGTGGCGC CTAGGTTGGGCACAGCATGAGGGCAGAGCGGAAACCTTGGCGAAATCCCG GCGAAGCCTGGACGTCGCCAGCTCTTACTTGACGCAAACATAGGGGGATT CAGGAACTCTCTTTACCGCATTTGCAATTAATTTGCTGCAAATCTAAAAT CGTTCCAAGCACAATGCTCACTGCATGGAAAAACCCAGGGGTAGGTCTCCCCCGATCAGGATGTTTTCCCGTGCCCTUTGTGCGGGTGCTGCCCCCTGCG CTGGTCAGTGAGAAGTGTCCCTCCACCGACGACATCAAACTTCCCAGGTC CACGUTCTCTGCTGTCCTGGACGAAAACTCATCTCTGTGAATCTCCCGCC AGCTCCGCGGGAGCCTTCCAGGGCTGGAAGGACGGCCGTCCCGTTCCAGG GGGCAGGTGCACGCTTCCCAAAGCTCCGCGTCCTGCTAGGACGCTCAGAC GGCATCACCCACAAACCCCACGAACTGTTTCCCTCGAGGCGACAGGCTCG CCCTTCTCCGAGAAAGCAGCCCGCACACGTCAGCAAGGGGCCAGCTGCGT TTGTAACTCAAATGGCCACATAGAGTTTGTCCTGGAGGCACGGGGTCTGT CTGGGCCGCACCACCACACGCAGAATATGCTGGGACACGCTCCGGGGT CCAGCTTCATGGAATTAATAAAGTTTACTGCTTCACCAAGTACATTCTTA AGTGTAGCTGGCCGCCAGCCTGGGCGTCCGCTCCGAGGCTGCCTCTCTGC CTGGAACCCTTGTGCTGGGGGACCCTCTCTCCAGCCCCACCCCAGCCCCG AGCCCAGGCAACATCCTTCTTGTAAGACACCCGCTACCCTGCCCTCCCGC TTCTCCTTCTCGGATCCAATCTCCTCCGCTTCTAAGCTCTCTTGAGGCT Contig 107 (550 bp)

TAACCCACTGACCGAGGCCAGGGATCAAACCTGCAACCTCATGCTTCCTA GTCGGTTCGGTAACCACTGCGCCACAACGGGAACTCCTTTGCTTTTGTTT TTAGGATTTCACATACACGTGATAACGTGCCGTATTTATCTTTCTCATCT AGTGGCAGGATTTGCTTCTTTTTTTTTTTTTTTTTTTTGTGGCTGAAAATCAG TCCAGGATTATCTTCTTTTTCTGTTCATCTGTGGAGGACACAGGCTGCGT CCGTGTGACGCTCTGCCGGGAATACGGGGGCCGATCGCTTTCTGAGCCAG TGTTCTCATTTTCTTGGGAGAAGTACCCGGAGTGGAACGGCTGGGTCGTC CTGCAGTTCTGTGCTGCATTTTTTGAAGACGCTCGGAGCGCTTTCCACAG TGGCTGCACCGACTGACATTCCCACCGAAGTGCACGGATTTCCCCATCCT TTTTCCACGTTTTCCCCGCACTTGCTATTTTTGCCCTGTGGATGTCGGCC TCTCCGTCAGGTGTGAGGGGAGTCTCCGTGCGGCCCAGGCGAGGAGCGAC CGTGAGCGTCGTTTCACGTTCCTGTTGGGCCACCTGCGTGGCTTCTCCGG AAAAAGGGCTGTTCAGGCTTCTTGCCCATTTCTCAGTCTGATTGTTTGGG GGGTTTGCTGTGAGTTGTGAGTTCCGCACGTATGGGGGGCATCAACC CTTTATCAGCTATGCGATTGGCAAGTCCGTTCTCCCATGTTCCGCCGGCC GCCTTGGCACGTGTGGGCGGTCTCCTTGGCTCTTCCTTGGTGCAGAAGGC TGTTTTGATGTCAGATGCAAAAATCCATTGCCAGGGTCTGTGCCGAGAAC Contig 110 (306 bp)

CGCCACCTCAATCGCCGGTTTGTTCTGCAACACGGTCCAGATAACCAGCG CACCTAACAGGTCGAACACTGCCAGAACTGCGAACAGCGGGCTGAAGCCG ATGGTGTCAGCCAGTGCACCGACAACCAGCGCAAACAGCGTACTTGCCAG CCATGCGGACATCCGGGTTAAACCGTTTGCCGTTGCCACTTCGTTACGAC CAAACACATCGGAAGAGAGCGTAATCAGCGCGCCAGACAGTGCCTGGTGG GCAAAACCACCGATACACAGCAGCATAATTGCGACATACGGGTTGGTGAA CAGGCC

Contig 111 (800 bp)

TCCTGGCTCTGTAAGACCTTGAAAACACCTCATTCCTCTGGTCTTGGCCT GCTCTTCGGTACGCCAAGTTGCTGAGACTGATGTGGGGATCAGTGGGGAG CAGGAATCTTTCTGATTCAGCCGTTTCAAAGTGTCCCAAGCAGAAGCTGT GATGGCAATGCCAAGGCTATCCATGGAGGTGGCTGTGCCAGGGGCCCCAT TTCCTGGGAGCCCATTCCAGGAAAGGAATCTTGTAGCCCCAGGCTCCAGC GAGCTGTGGATGGTAAGCAGGTGGCCCAAGTCCAATTTATGTCTGTGGTC CCAGCAGGGTGCCCAGGAGGCCCCTCGTAACTCTTAAGAATCTTGGTCTG GTCAGCTAAATTGTATGACCATTGTACTGAGCACACATCCCGTTTAAGTA GAATTTTCAAGGATGACTAGGAGTTTGCCACCTGAAGGCAGGAAGGGCAT TCCAGGCAGAGGTACAGAGGTGAGAGGGAGGCTCTGACACTTTGGGCGT GCAGGGGGTTTGATGTGACTGCAGCTGGCACACAGTGTATGCCCAGGCCT GGCACGGCTGTGTTGGTGTTTGGAGAGGAAGGGAGAGGTGAGTTGAGCCC AAGGTCTTCCAGGCCAAAAGACTGAAGGTGACCGCGGCTGTCCGGGGCTG GCCCGCAGACCAGGAGGAGCAGGTGGGAGCTGGCTCTTGTTCCGGGGAC Contig 112 (3062 bp)

CACACCCCAGGAGGAAAGACCCACACACTCCTGATGACAGCTTGGCTC
GGGGCTGGAGCCCCGAGTTATAAATGTCCATCACGAGCTGTGTTCTGTCA
GAGCCATCAGTGGGAAGGCCAGCCAGCTCAGCAGCCCAAAAATGAAGAG
CTAGGTCTGGGATTGGGCCCAAGCAGAGGGCACAGGAAAGCCACATAAAC
CAGGCACCCAACCCCCTGTCATCACCAATGTCACATTCAGGTCACACC
CCTGGTCTTCGGGGGAGGTCCCCTAAGATCCGGTGGAGGGGAGGAAAA
GTCTGACTGGATTCCTTGACAGGTGTATCAGCGAAGGCCAGGAGGAGTG
CTCGGGCACTGCCACCTCCCAGGGGCATGATGGTCATGGACCAGATGCA
GTTATGGGAGAACCTCCCCCGTGGTCAGAGCTCTGGGCTGATCCTGACCTG
CTATGCATTTCGAGTGGAAGGAAAAGAAACATACAACTCCACCCCAGC

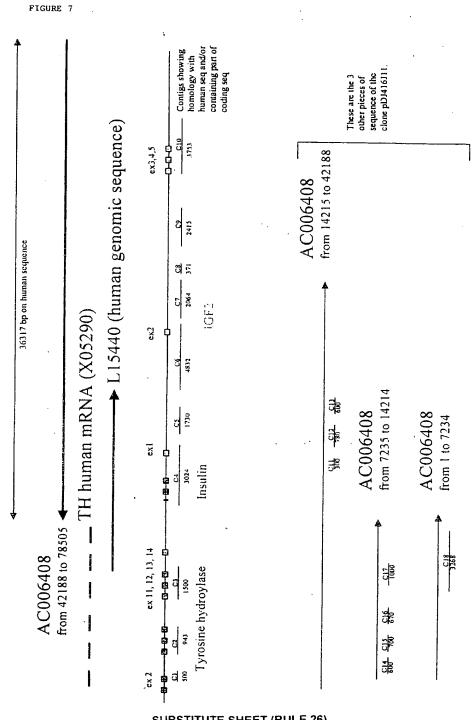
AGCTTTAGGCTGTTGGTCTAAAGGTCCTGCCTCCTGGAAGAGACACGCCT CTGTCAGCGGACACTGCTAAACCTAAAGGAAGAACTGCCACCTGGTCACG GGACTTCCTAGGCCAACCAACCTACAGGTGACGGCCCGGAGCATCACGAG GAGGTAGGGGACGGGAAGGGATGCATTTGCTGCTCAGCGGATCCACTGGG GCGTTTCTGGAGCCCCCACGCCCACACTTTACTGCAAATGCACAAGCCCC AGGCAGCAGGACAAGTCACAGTAGCTCTGGGTTATCCAAGGAGTCAGGGA CCTACCTGGAAGAGTCTAGAACAGGTGACAGAGGAGGAGGATGGTAC CAGCAGTATAGGGAGAATCAGAAATCTGACCCACCCTGGGGGCCTGACTG GGGCTGGGCCACGGGAGTTATGGGCCCCAGGTAGCATCAGAGGCTCCCAG CTACAGGCACAAGCAGCAACCACAGGAGGATCCAGGCCAGGGAGCATCC AAGAAGCAGCAGAAGCTCCACCTTAGGTACAGTTCTGGCACCTCCAAGTT GAGAACATGTCCTAGACAGTGCCTGACCCCAACCCAATGGAGTGTCTCTGGG ACTAGACTAGGCACGCCATTTTGGTCCCAGGTTGCCCCATCTGTACAAAG GGTGTGCGGCCCCCAGGGGGACACAATGAGCTCCCATGGGAAGGGTCTTC CGARTCTCCTTAGAAGCAGATGTAAGAGGTGACGTCCAGCTTGTGCCTGG GATGTAGAAGTGGAAAAAGCACCCCTCCCCCGACAAGGATGAAAGCAAGA GGCACAAAACAACCTGAAATTCCCAACGCCCCTGGAGATCCTTGGAGAAC TGGGATTCTCCACCTGTAGGGGCACCTGTGAGGAGGGCTGTGTGAGCAC CTGCTGACCTGGCACAGAGGATGCCCAATACTAAGAAGCATCAGCTAAAA GTCTCCAGGAATTCCTGGAAGCTGAGGAAGGGCTCACGAGAGGGTACAGA AGCCCTGGGGCTATAGATATAAGGGACGTGCACACCCACTTGCAGGTCCC CATGGACCCCAGGGACATTCACAGTGATGGGCAAGATTCCCAAAATGCAC CCCTTGTGTGGGCCTGGTTCCGTGGGTCAGCAGACACCACACCAAAGG CACAAAGCACACCCTCAGGCTACTCTCCTCCCTCTCCCTTGTGGAACA TGAGCCTTGAGATGCTGGGGGCACGTGAAAAACACTGTCACACTTAGGTCC TGGTGAAAACTGACTGCGGCCAGCGGAAAGAATCATAAAGACCCTACACC CACACACAGCCTTAATTACAGCTGTGAGTGGGGCTGGAGCCCCAAGAATG TCTACACCCATAAGACATAGCGTTAATCAGAAAAACAAGAACAGCCCCCAA CCCCACCACGAGGCTGACAACTAACAGGTCATGTTGGAATATCACTGGGA ATGTTCTAGGAGTGTAGAAAGACACACCAACTAGGGCATGATGCAAAGAT AATACTTCAGCCTGGGAGTGGATGTGACACAGGGAAAAGCATAAAGTGAT GGCAGAGGACTTTGATGTCAGTGATGGAAGCCACAAAAACTTCTAGCTTA GCTCCATTCCCAACAAGATTGACTGCAAACCCCATGCTAAAACAACAGCA AAAAGAAAGAATCCTCATTTCCAGGCATAAAATTTTTCCCCCAGTCTCTG CTGTCCTCCATAAGATGTCTGATTTCAACAGGAATTACGAGCCTATAAGA AAGGCAAGAAAAACTACACACTGTCAAGAGAAAGCCATCAGAATAACCA GACTCGTAGCACAGACACTGGAATTGTCAGGATATTTAAATAACCGTGA CAAATACATTAAAGATTCTAATGAGAAGGGGGTAGACATGTAAGATCACA TAGATTTCAGCAAAGAGATGAAACTCGAAGGAAAATTAAATGGGAGCCCT AGAGTGAAAAACACTGTAGCAGAGAGATGGGTTCATCCGTAAACATGAC ACAGCTTAGGAAAGAATCAGTGAACTTGAAGACAGGGCCACAGAAAATAT ATAAAAGAACAAAGCATCCAAGAGCTGGAGGGTGACACTGAAGAAGAGAG CATAGGCATAGCTGGAATCTCAGAAAGAGAGAAAGAAATAACCCAAGATG TAATGGATGAGAATTTCACAGAAGCGTTGTCAAGCAACCAAACCATACATC CAAGAAGCTCAGAGAACACCAAGCAAGGTAAGTACTGTAAAAAAATAGCC CGAGGTATACCTCATTCAGGCTGCTGAAAATCCATGACAAAAGAAGTCTT GAAAGTAGCCAGAAACAGAAGGCGTGTTCCATTCAGAGGGAAAAGACACC ATTGTTGCCAGAAACCAAATAAACCAGGGCTGAAAGGGTAAAACTTTTTT TTTTTTTTTTTTTTTTTGGCCATGCCTGTGGCATGTGGACGTTTCCCGA TCAGGGATCAAC

Contig 113 (1300 bp)

AGGAGTCGAATCGGAGCTGCAGCTACAAGCCTACCCCACAGCCACAGCGA CACAGGATCTGAGCCATGTCTGCAGCCTACACCACAGCTCCCGGCAATAT TGGATCCTTAACCCACTGAGCAAGGCCAGGGACTGAACCCACGTGCTCAT GGATACTAGTTGGGTTTGTTACCACTGAGTCACAGTGGGAACTCCTTTAA TTTTAATTTTTGAAGGTTCAGAACTCTTTAATTTTTTAGTGAGGTATAGA TTATATTACGCACCATTTCTTTCTGACTTCGGTGCACGGCTTTTCAACAA ATGGGTGCTGGACCTGCTGGGTGCCTTCTTCAAATGAACCACAAGCCCTC CCTCGCGCCGTATGCAAAATTTAACTCGAGGGGCTCATAGACATAAACGT AAACTCTAAAGCTATAAAATTTCCAGAAGAAAACGTAAGGAAAACCTTTG GGGTCTTGGGCAAAGATTTCTTACCCATGACAGCAAAATTACAATCTACA GAAGAACTGGTGGCCTTTATCGGCATTTAAAACACCTGCCCTTTGAATGA TGCTGTCGCAAAACCGAACATGCAGCAAAACGGATGCAACTAGCAGGTCT CACACTCAGTGACCCACGTCAGAAAGGGAAAGACACGCCACGTGACATCC CTTAGATCCAGAATGTAAAACACGGCCCCCGTGAACCGACCTCAAGAGAG AGACAGACCTACAGACGCAGCAAATTTGGGGTTGCCGAGGGGGATGCCGG Contig 114 (3000 bp)

TGTGAGACCCCTTGGCGGGCCAGGACCCCCAAGGTGACCGAAGGCCTCA GCGCCCCAGCCGCCCATCCCCCTCTTTCCCGACACAGGATTTTTTTCC CACCAAGCTCTGTTCCCTTGGTCACGCTCTCACTTGAGCAGCCTCAGGGT CTCCCGGTGCCTGTATCCACGACAGCGTGACCTTCTTGGTGTGTCAACCC AGGACCCCACGCTGGCCAGCCCACGCCTTCCCAGAGCACCCCCGCCCATCC TCAGAGTCCAGAGGAAAGGCCCCCATTGACCCCAGAAACCAAAACGCAGA GACTCTGGGACGCCAGCAAGAACGTACACTGACTCCCACCTGCTTCAGGC ACCCAGGCAGGGGTGGGTTATGAGCGACCCCGTGGAAGGGCCTTCTTGTC CATCGAGGGGCTTCCAGGGGGCTCCTAGACGGGGATGAGTGTGGCAACATG TEGECGCATTACAAAAGACCCTGCAGTGCTGCTGGGATGGGTCCCCCGGC TAGAAAAGCAAAGGATTCCAGCCCAGTCGAGTAGGAGGCGGCCTCGGAGG AGGGGACGCCCGGCCGGCTGCAGCCGGTGCGCCTCCGGATAAGCTCCTA AGAGGCCGCGTGCCCCATGCACGCGCGTGCACACACTCGCTGCCCGAGGG TCCTTCAGCACAGACCTTCTGGGGACGGAGGACCTGGCAGGGGTGTGGCT CTGGGGAAGGGGTCTGTCCCAGGAACCCTGTTCTGGATTTGGGGGTGGGC GTGGATATCCCGTCCCAACCTACAGAAGGGGGGGGCTTAAAAAGAGCCCC TTTGGTGTGAGGGGCCAGCAATCCTTTGGCTTTTTCTTGGCCCACTTGGA GCTTGACGTCTGGTCAGTGACTGGGAGCCAGGGGCCAGAGGGGGGCAGCCG GGCTGAGGCAGGTTCAGGCCAACCATCTCTCGGCCACACTCCCGAGGTCG GGCAGCTACGGGGCCCCCAGAGACACAAGCCCCAGGGGTCCTTCCCCCCC GCCCCTGCCCAGATCACCAGGAGACCCAAGCAGCTCTGCCTCCCGTG CCTGAGAAATGCCCCATCTGGGTACCCAAATCACCCTCCCAGAAGGTAGA TCCCAGGGGCGAGGGGACTCCGTTTGGGGCACAGACGGAGGCAGAGCGGG CTGATGGATTCTCCCCCGGTTCAGGGATGCTGGCTGCCTGGCCTCCAGGA GCCGGCGGTGCCATCTGATCTGATTAAGGCCTGCACTCCCAGCTGGGCGG GCACAGCCTGGGGGGCTCGGCGGGGGGGAAGAAGGCGCTGTCGCCCCAGC CGGTCAGGCTCGCTTTCTTCTTCATTTCCTCTCCATTAAAAGTGTCAGAAC CATTTATTGATTTTTTAAATCAGGACGTGCTGTCCGTGACACAGCAAAGT CAACAAAATCAGAGCAAAGAGAGGCCAGGGCTGAAGCCCCAGAGGGCGGC GCCTCCAATCCGGGTTGTGCCCCGGGGCTCCAAGCCCCTTCTTCTTCTGG GGTCCTGGGCGTAGTGGCCAGGGCAGAATGCACCTGCCGTCATCCTGGGA GGCTTGGCCATCGCTGGCTTCTGTCTCATGCGCACCGTCGTTCCATATC TACGGAAACAGCTTCGCATTAACAGGCÁGGGGAGGCGGTTGTTTCTCCTT TATCTGCCCACCATCGGCGCTGGGGCCACGTGGAGCCCAGCCGGCTGACT TCCCGCTCGCACGCAGGGCACTGATTGCAGGAACGAGGACATCCAGCCCC CGCCTCTCAATGCCCCGGGTGCTGAGAGCATTTCGCCCAAACGGCTTGGG GGCCTGCCCGTGTCTGCCCGTGGCCTCCAGCACCCTCGGCTGCCAGGCTG CTCTGGAGAGGTGCCCGGGGGCCGAGGGCCAGGGGCACCCTGTTCTGCCC CACGTCTCTCTGTCCTGCTGAAAGTTCCACCAGACGCGTGCTATACCCTG GGAGTCAGGAGGATGGGGGATAGTTGGGGCTTGACGTCTGTTTCTGAAAA AACACCGTTTTCCCTGAAATATATATGTATTAATTTTTCGTCAAGATAAA ACTGTGTATAGTTTTTCGTGATGAGAAACGCATCCATCTTCCTTAGAAA GCCTGAAGAGGTACAGGAGCCTATAAAGGACAAGATGACAGATGCCTCTA ACGCACACCAAATGTGCGGTGGCCCCCAGGGGACCGCATAGACGGGGGGG CTCCAGATGGCCACCGTGTGCGAGGGACACGGTTCAGGGTGGCAGAGTAT

TCATGGAAGCCCTTATCACAACCTCGGATCCAAAACCCACTGCGCGAGTC CAGGGATAGAACTCGCATCCCCACAGACCCTATGTTGGGGTCTTAACCAG CTGAGCCACATGGAAACTGGGTAATCTATTTTTAGATGTTCCTAGGGTTT TTGGCCTTGCCTGTACGTGGGGACGCTGCTGGGCCAGGGATCAAACCCGC GCCACAGCTGTGACCCAAGCAGCAGCAGCACCGGATCCTTAAGCA CGAGGCCAGCAGGAGCCCCTGTGTTTAGATTTTGGTGAGGATACTGCGT GGGATTCAGGATATTCACTTTGGGGCTGTTGGAATTGCCCGTCGCTGTTT AAGCAAAGAGAAATCCCTTCACTCTGTGTAACTGTGGGGAAATCCTTTAG TCTCTTGAAACCATTGCGTGTGTTTAAGAGTGGTAACTCTGCCACCATAA ATGCCCAGACCAGCGCCTTCCTGAGATCCGCTTTTGTTGCAAATATCTGG TTTGAATGUTTTGATUGCCCGCACCAGACCAGGGTGGGCGGACGCCGCCG GGGACCCGACGTGACCATCGTGCTTCTGTATCCGCCCTTTCTCCGGCACG CGCCCCCTGGTTGCCTCTGGCTGCTTTTAGTGGAGGAACTGAAGCCTCGC CACCCAGACCCCGAGACCGCAGGACCCACAATGCTTCAAACACCTGCCCT CTGACTTTACAGGTCAAGTTCGCCAACGCCGAATTTGCACCGATTGGCT ACAGAGAGCACGGTGGCGCCAAGCCTCCACTTGGAGTTTTATAAGGTCTC CCTCCAGCTCGCAATGAAAATGAGCTGTGATAAGGCAAAGACAAAATTAG TATGAAATCCAGATGCTTCATCTACAATACAATGACCGCGGGATTTGGGT CTGAGCGACTGAAATCAAGGTGGGCTTCCGGAGGGAGGCTGTTAGAGGAA AGGCATTCACGCAGCCTCAGGTCCGAGAGGCTTCCACACCCCTAAGAGGG CTGAGACGGCAAGTAGGGACCAAGCCCCGCAGTCGGGAGAGCTGGGCAGG AAGGAAGTCTGAGGTCACCCCCACCTGGGGAGGAACTGCCTAGAGAAGCG GGGGCGGGAAGCAGGGGATGCCCAGTCCCAAGACAGGGACAGGGCGGAAA GGGCTCTCTGCAGGCCCTCAATGCTGCCACAGTGTCCTCGTAAGAGGGAG SCAGAGAGAATTGACACCGGGGAGACCACGGGACCACGGAGGTGGAGACC GGGCTGCCCGCGCGTGCCAGTTGCTCCCGAAGCCGGCCCCTCCCCCAGAG CCTTTGGGAAGAGGCGCCAACCTGCAGTTCTGCTACTCGGGGACAGGGAC AGGGACAGCCCCTGGAGCCGCCTCTTAGGGGCAGCATCCCCCAGAACCT TCCTTAACAGACCATCTGGAGAGAGATGGGTCTGGGCTGCAGCTCCTGGA ACTGTTTTGCCCACCCGGCGAGCACCAGTGGGTGCCAGCCTGGGCTGCCC AGCCTCAGGGCCGGGAGGGCTGAGGGCACTGGGGCCCGGCTCTGGGACT CCCTGCCTCCTGCCCGTGCAGGACAGCCACCTCCCAGCATCTGCTTCCT GCCACCCACATCCCCAGGACCGTCAGCCCAGGCATGCCCCTGGCGTCGGC CACTCACACCACAGGCCAGGAACCCAAGGGGGCAACACAGAAGGGCAGTT GCCATCTGCAGATGGAATGGACAAACTGGGGTCCGTGATGATGGCAGGCT CTGGGCGCCCGGGCTGGCAGGGGGGCCAGGACTGTGCGGCCATCACAGGA AGGGCATGACGGGGTGAAAGCAAGAGTGGAAACCTCTGCCACCCGCCTGG



SUBSTITUTE SHEET (RULE 26)

FIGURE 8

Contig 1 (1040 bp)

GCCAAGTGTGGGGCTCTGCTCCATCCTGGCTCGGAGGTCCACCCATGCCAAAGCCTGGGG TCCTCCCACTGAATATTTGGGGGTCCACTCGTGCCAAAGGCTGGGTGTCCAGTGTGCCAA CGGTACATGGAAGCAATGTCTTCCCAAGGACCGTCCAAGGTGTGGTCAGGCCTGGACAGC TGTGAGTCCCTTCGGGACTAGACTTGGTGGCCGAACCCTAGGGACCGTGCCCGAGGGCCC CCACGAGGCCAGGTGTTTGCCCCAGGGACAGAACGGCCAAGGGTGGCCGAGGGTTCTTTT CTCTTTGTCCCGATCCTGAGCGGGCAGTGTCCTCGTCGGTGGGGTGCTGCGCAGCCGCAG CAGGGCTGAGAGAGCCCGGCTTGTCACTAGGGCGCCCCCGGTGAGCCCAGCCGGCATGCCG TGTCCAGACGTTGGATGGGGCAGCGAGGGGACTGGGGTGCCCCAGCCCCCGTGGGAAGCC CGCCCTGTGGAAGCCGCTGTGCTCGCCACAACAAGCACCGTCGACTAGCTGGTGAATCAG CGCCCGTCGCCCGCGTAATCCCAGGCGCTTTCTGCCCAACCTGAGCCCTGACCCCACACC CCTTGCGACCGCTCCGTGGACCCTGGGGCGATGAGGTGAACCGTGGGCTTGGCCATCGTG GCACCCAGTGGGGGCTGGGCAGGGAGCCGCCTCCACCTCCGCCCTGAGGGGACGGGACTC GGGGGAGAAGGCCCTCTTTGGAGAATTCCAGGACGGGTGAGGAACGTGTGCTGGACCGGC CGGGTCGGAGGTGGGCCTTG

Contig 2 (9234 bp)

GGCAACCAGGGGAAGATGGGGAAGCGGGGTGCAGGGGGGTTTGCGCGGGCCAAGGACCAC TGGCTGGCACCTGGGAGCCTGGCGGGTTGAGGTCCGGGCTCCCAGGTGCCCTATAGGCA GGGCAACATCGGCATGGGGGTGACAGGCCCGAGCTGGGGTGCGGAAGAGAGGGGGGGA GCCAGGCATTCATCCCGGTCAATTTTGGTTTCAGGTCGTGGCGGCTGGTCAGGGGGGA CTTGGAGAGAGGTTCGCCCCGGGGCCTGGGGGCAGCGGAGGTGTAGCTGGCAGCTGTGGGC TCTGGCCTCCTGCATCCGGAGGTTCTGGGGAGCGAGGGCGAGGCGAAGCGGCTGAC CCCCCGGCAGAGTGGCGGCGGACGACAGGCAAGGCGGCCAGAACAGGTGACACGTCTCAG GGGGCATTCCAGGCCCGGGTCGGACAGGACCCGTCGGGGGTGTCAGGACAGTGGGGTCCC CAGCCGCCACTTCACCCACTGCAATTCATTTAGTAGCAGGTACAGGAGCGGCTCTGGCCG GGCCTCTTGAGGCCTGAGCTGGAGCCTCGAGGGCCGGAGAATGGGAAAGAAGGTGCAGTG TGCCAGACAGACGTCACCTGGAGGGGGCCACGGCCGTGGGGACCGGCCCCAGAGAGATTTC GCCAGCAGGAGGCTGCGGGGCCCAGCCTGCGGACGTGCGTTCCCACGCAGCACTGCGG CCCAGGGGCTGGCGGGCAGGGCCCCGGTGTCCTTGGTGGCACTGTGCGCCCTCGCCCC TCGCCCCTGGGACTGGCACGGCAGACAGGACAGCACCCAGGGGAGTCAAGGGCACTGACG GGGGACGCAGGCAGGGGCGAGGCGCGAGGCGAGGCGAGGCCAAGGCGGGCCT CTGCGGGTGACAACTGAGCACATATGGGTACCTTTGCGCTCGCACCGGAGACAGGTGAGT GTCTGGCCCCGGCCTGCCGCCCTCCCGGCCCACTGCCTCTGCCCTCCCCCTCGACC AGGGCCCTCTGCTTCCCCACAGCCTCGTCTCCAGTGGGGGTGGACACACTGCCAGCACCA CAGGCCGGACGCCAGGATGTGCTTGGAGGGACATGACACAGTCCGGTGTGACGGAGAGGG ACAGACGTGACGCCGTCCGGCCTTCCTGGTGAGCGCAGGTCCAGGCCTTGGCCCCCAGGC GCCCCGCGGGGGAGCTGCCACACCCAGCGTCTGTTCCTTTGCCTTCCTGAAGGAGCACGT GCATGACTGCTCTCTGGACCCCAGAACCCTCAAACGACAAGGTGAGGCAGGTCCCGC CTCGCCCCACACGTGGAAGGGGCGTGGGCGAGAGCCGGGCGCTCACGGTGCCCCCCTCCC CCTGCAGAGATGGTGCTACCCAGCTCATGCCTGGGCCTTGGACCCGGACTTCTTCAAGTC CTCCTAGCTCTGACTCAAGAATATGCTGCATTCTGGAGCCACTACACTACTTGACTCAGG

FIGURE 8. CONTD.

CTCCCGGCTCACGTCCTGTCTCTGTCCTCGTCCGCAGGTTGAGCCAAAGGAACAGACGTC CCTTTTTTCCTCTGTCTTTTCTCTTCTCTTTCTCTCCTCTCCTTTGCTCAGAAGACTCGG TGGTTTGGAGTCCTGGAACCAGCCCCGGGTCTCGGAGCGGGTGTGTGAGCTGCCGAGTGG CGACCACCGAGCAGACCTCAAACGCTGCACTGAGTGTCCATCTCGTCATGTGCCCCTCCT CGCCAGGGCCACCCCAGAGCCCTGGACTCATCAATAAACTCAGTTACCGGAATCTGTCTC AGGGGCTTTGCAATTGGGCTGGGGGGTGCGCCGGGCGAAGGGGGGGATGAGATGGGGAACAT GCAAGGAAGGCCCTGTGGGCTGGGGGACACAGAATGGGTGCGGAGGGGGCTCACAGGACT CGGGGGGTAATGAACGTGGGGCTGGGCGCAAAGGGGAGTGGGACGTGGGGATCAGGGCGG GGGGCCTGGAGGATGCAGGGTCCCTGCAGGGAAAGGGGGCCGAGGGCGTGAGGCATGTCC TCAGCCCTGAGAGGCCCTACCCCACAAAGCACAGCCTGCGCGCGACCTCCAGGCCCCCAA CATGCCACCCAGGCTGGCCACACCACTGGGACGCCCATGGGCGGCCACTTTCATCAAGAG CCTGGCAGGCCCTGAGTGCTGGGGCTGGAGGGCACAGAGGGTCCCCCTCCCCTCACGCTTT GCGGTGCTGGGGCACCGCAGGAGTGCCCAACAGGAGACCCCAGGAAGTCTCCTGGGCTGC AGUGAAGGCAGGCTAGGGGGGCGGCCCACCAGCCAGCTCAGTAGGCAGGTGGCACT GGGAGGCGGCAGAAAGTTGGAAAGGGTGGACTGGGCACGTCACCATCTCGTGGCGGCAGC CCCGGAGCCACGGCCTTGGGTGCACTGCAGCCCCCACGGTTGGTGTCCCGGTCCCAGGCA CCTCCCAGCCCCTGGGGGCCTGGCGTGACGCTGGGAACGCGAGGGAGCAGGCCTCGGAAA CAGGGCTGGGTCCTTGACCCCTTCCTCTGCTCAGGGCAGTCAGGAAATGCCTAGGGGGCC GACTGACCGAGAGAGATAGCGGAGGCCTGGGAGACCCCGCGCTCGTGCCGTTCCCAGCG TCCGGCCCCGTGGCCTTGGCTGGCCTGGTTTGGGCCCCATGAGCTCACCCCCCGCCCCC TGCGTTTGGTGAAGCCCCTGCCCAAAGCACCCTCAGCGTTTCCTCTGCGCGTCCGGCCGC CCCCGGAGGCTTTCCCAAGTCCACGGGCAACTCGCAGGCGAGCCCACTCCACCTCCATCA CGCGGGTTTGGCCAGCGGCAGAAGCACTCGCCCTTCAGGCGTCAGGAGTTAAGCCCCTCC AAGGCCCGGTGCTAATCAGCTGCCTCTCCTGGAGCTTCGCAAAGCGGGCTCTCAGAGCCC AGCTTCCCGGGGGCTCACCGTGGTGGCATGGGCACCACAGGTGGCCGGAGGGGCACCGAG CACGACGGGGCTGTGGGGGGGGGGGGGGGGGGGTTCUTGACTCCGAACCTCTACTGAGGCACACAGAGGGCACACGCCGCTTCCAGGGGGAGTCAGCCTGCGAAGGGCAGAGGGCTGTAGC CTCCCGGTCACGCCCTCGCCTCTGCCCTGGATTCCTCCTGGGGGCCCGCGGCTCGTCGGG CAGCTGAGTGCCCCTGGATGGGCGTAGGCTGGGGGGGCCAGGGAGTGGGGGAGCCCCGAGG CCCTGGGCCCACAGCCCTGTCTTGCCCCACACACAGGGCTGTCTACACTGGGTGCCCACT TGCTCTGCTTCTAGGCTGTTCCCTGGGCAGCTGCCTGGAGGGCCGTGGGCACAGTGCGGG CAGCCAGTGGGGAGGCCGGGGATGGGGCCGGGGATAGGGACCCCTGCCCCTGGGTGAGCC CCACCTGGGCTGGGAAGACACCAGCGCCCCTTCAGGTCCATGGACCAGGGGACCCAG GGTGGACTGTGTTACCTTCAGCCCAGGCCAGTTTCCTGCTTGAGAAAGCCCGGGAGGGG GTGCGGGACAGGCCCCGGGCCCCCACGCAAAGGCAGTTTCGCAATGTCCCTGCGCTGACT GGGCACCGGGCTGCTGGGATCTTGGCCCCTGAACCTCCCCGGCCCTGCGGCCAGGGAGG GTTTACGCTGAGTGACAGCCCACGGAAACCTGGACCCGACATGTCTGTGTGTCCATGTGT GTCTGTGTGTGCGTCCACCTATGCGTCTGCGTGTGTCCATGTGTGTCCACATATCTGT CTCCACGTGTCTGTGTCCACGTGTCTGTGTCCACGTGTGTCCACGTGTGTCCATGTGT CCGTGGACCTGTCTCTTATACACATCTCAACCTG

AAGAGCAACGTCTGAGCTAGCTCCACGCGTGGGTCCATCTCGGCCCCAGGTTTAATGAGCC ACTTTCAGGCAGGGATTGCACAGGAGGCAGGGTGGGAAGTGGCTCTGCTCAGACCCCTGA ACAGGGTCTGGAGATTCTCCAAGGGCACAAAAGAACGGACGATGCCCCTGGGGTCAGCGA CAATGCTCCCTGAGAAATCTTGGCACACAGGGCTGGGCCTGCGAGGTGGCCCCTCGCCCC ACCCCAGCCTCCTGGAGGACAACCCTCCCCCTGCTCCCAGAGCTGGGGGGGCGCCACACGT GGGGCACAGGGAGCATGGGCCCGATTCCAGGCCTGGGCTCCCTCTCGTGTCCAGGATCTC CCCGTGTCTTGTCTCAACAAGCCCCTGACTTGGAGGCCCCAGGGTGACCCCTTAAAGGGG GAACAGAAGGTTCTAGAAGGAGCGTGGCCAGCTTTGGCTTCCCTAGGGCTGTGGTGACCA ACCAGCGGGGCCCCTTCCTGGAAGCCCACCTGCAGGCCGGCTTGCTGGGAAGGGGCCTGC TCCTCGCCGGCCCCACCCGCCCGGGGCCGTTTCCTGGAAGCGGTCACTGGATATTTTGTT CCTTGTCAGCGCCGAGCTTGCATAAAGCAGACACTGAUCTCCTTGTCCTCCGGGAGCACG CGCTCCATCACCGAACACCTGGCCGGACACAGGCGGGCAGCCGGGCCTGGGGGAGCAGCG TGCAGGCCGCCCCACGTGCCCAGGCCCAGCGGTGCCCATCCTGCAGGCTGGGAGGAGGC CTTTGCAGAAACCTTGGCCGGCCTGGATGTCTTGCTGGGAGAGCTGGGGGAGAGGGAAAAC GCAGGAAGCCGGTCCCCCGAGCGGGGTAGGAAGAGGCCTCGGCCCTGGGAGGAGGAGGA ACAGGGACGTGACCTGCGGCCCGGTCCCGGGCCCAGGGGGGCTGGAGGGCGCCTGGTGG GTCAGCGCCACTCAGAGCCCTGCCAGCAGGGGGCCTGGGGCACGGCTGCAGGACAGAGCTC AGGACACAGATGGGGGCGAGGACTGAGTGGGGCACCACAGATGCTCCCAGGAGGTGGCCA AGGAGTGGCCTTGGGATCCCAGGATGGCCCTGGTCCCAGAAGATGCCGCAGCCCAAGGGA CCAGGCCAGGGCCGCAGGGGCCACAATCTGAGCAGGGCTCAGGCCCAGGGCAGAGGCCC CCTCCCACCCAGCCCTCCCTGGGCCCGCCTCTCC

GTGCAGGCAGTGGGCTCAGATGGGGCAGACATGAGACCAGGTCCAGGGAGAAGCGGGCCC TCAGGAGCACACAGACCCCCACCACGGGCTCCCCCAGGTTGGGCGGTGACATCAGCCCTG TGTCAACAGCAGGAGCTGGCAGCTCCCCACCGGGGCTTAGGGAGCGGGGACCCTGAGCCA CCCTGCCACCGCCCCACCGTGGCCCACACGAGGGCCCGCTGCTCTGGGTCTGGGG CCNAGGCCCCCCAGGCGCCTGGCACTGTCTGCCCCTCCCGCTGGCTCTCCGTCTCCAGTG GGCCAAGCTCTGGTTGACCTCCATCCTGGGGCTGTGGGCCTTTGGTCAGTGGGGAGGGTC ATGACACCCAGCCAGCTGGTGACAGCCCTGGACGTGCCGGCTCAGGGCTGGCCTGC CCCTGCAGCCTTGAACCCCTGTTCTCTGGGAGTGGGGGCGCAGGGGGGGCCCGGGCCAGGG TGAGAGACGAGAGCCTCTCTTCCCAGAACTTCTGCCTGCGATGAGGACCCAGCAGGGGCC TCTCCTCACCAGAGGGCCTCTGCCGGCTGCAGGGCCCCAGAGAGGCCCAGAGGGCTGGAGG CCGGGCCTTGGGAAGGCCGGACTTCCAGAAACCAGCTGCCCGCTCCGCAGCACCCCAGC GCCCACTTGGGAGGGGGGGCGCCCCCGTGCCCCGGGTCCACTGCTGGGGCCGCCA CAATAAACTTTGTCCCTGCTGGTTACTGTCCGTGTCTGAGAGGTTTCTGGAGCCTGGCCA CAATGGGCGTCAGGATGCGGCTGGGAGGGAGCCTCGCGAGTCAGAGTGTGCTGGTCTCGG GAGGGGGTTGGAGAGGGTGGGCGGGACGAGGGGCTTCCTGCACTCTGTCCCAGGGAAGCG GGGACCAAGGAGGGGACAGCCCCCGGTCACCAGGAGGGTCCTGTCCCTCTCACCCCCCGG GACAGGTGAGCTCCCCGGAGCCGCCCTTCTGGGACAGGACCCCACGGCCAGGCCACGGCC CCCCCCACCCGTGGTCCCTCCGTCCCACGGCCGGCCTGGGGGGCCCACGGGCCCAGGGCC CCCGCTCCCCGTTGGCCCTCCGAGGGTGAACGACCTCGCCTGGGACGTGGGGCAGAGGGC AGGCGCCAAGAGTGACCCCCTGGGACACGTGGCTGTTTGCAGTTCTGGAGGCAGCCGAGA AGCCCCTGCCCGCCGCCGCCGCCGCCGCCGCCGGCCTGGGGACCCGGCCTGGTGCCCCT CCCCCGCCCTGCTCAGGGGCCAGCCCTCTCTGGTTCCCAGGACGCCCCGCCCCGCAGG CGGCCAGAGAGTCCCAGAGTGTTACCCTCCCACGTGTGGGATCCTGTCATATGCGACAGC CTGGGACACTTCAAGGGTTGACATGCTATGCCTGTCACGGATAAATGC

Contig 3 (5347 bp)

AGATGTGTATAAGAGACAGGGGCTGGGTGGGAAGGACAGAGGGTGGGGCCGGAGGAAATG

GGATGCAGAGCCCACCGTGCACGCTCTGCTGGCCTTTGAGCCTCGCTGAGTCGCAAGAAG GGCATGGCTGGAGGGCCCGAGAAGCCACCCAGGCTTCCCGTGCCGAGCTGGGTGCTGGGC CCAGCCGAGCGGGCTTGACGCCACGCTTAGCCCTCCCCAGGGAGCCCAGGGTCGGAAGGA AGAGGCCGGCCGGAGGGCCGTGGCCGCTCAGGCTGGAGGGGGCCCCCCGGTCAGGATGGG CCCCAGACGTCCCCGCCCCCGGCCATCCGTCACGGAGCTGTCACCCAGGAACGTGCTCC AGACGTGCTTTCCTCCCGCGAGGCCCCGAGCAGGCTCCAGGCGCCCCCACCCCCGAACG CCCACGCACACCCTCGGTCTGCGAACACCCTGCCGTCATCCGGTGGCCCCGGTTCCCGGC GCCCGCGCCATCCGGGTGCCCCTTCCTCCCTGGGTCGGGGCCATGCCCTCAGCGGGCAC GCAGGCCTGTGCAGGTCTGTTCTGACTCTTCCCCAAAGACGCAGGCCGGCTGCGGGCGCC CCGACCTCGTCTGAGGCCCGTTTGTGCTCACTGGCTGTCTCAGAAAGGGGTGCCCACGGG AAGCGCGTGTTCCTTGGGCCGCAAGGCAAGGGAGCCCACCCCAAGGTGGCTGAGGGCAAA TGGCCCAGGGCCTCTAAGGAGTCCCTGGGGGGCCGGCCTGCAGCTTGAGGAGGAGA GCCCTGGCTCTGCTCCCCGGGCAGGTGAGCCCACGGCAGGGGGCTCCCCAGCAGCCTTG GCAGGAAGCAGTGAGGAAGGGGTGAGGATGAAGGCAAGGGGGCCTGCGGGGACTTGGGCA AAGCCCCTGAAGAACTGAGTTCCTCGGAAAGGCCGGAGCCTCAGCCGAGCCTCGGCCTC CGAGCGATGGAGGCGGCCCACCTGCGGCCCCAGGGTGCAGCTGTGCATCCGTCCCCCTCG GGCCTCCCCCTGCCCCCCGGCCACCACACTCTCCCCCTTTTGCCTTTGATCACTTGAGT GCGACAGCTTGTGCGGCCTGAGCCCCAGAGACCGCTGCCCCCTGCCGCCAGGCCCACGG GAGCGTCCACCTGGGCCTGGGCACTCATCCCTCCCGGATGAGGCCTTTCTAGCCT GGGCCGCCCGGGAGCGGAGACCCAGCCCCTCGCCCCCCTCCCCAGTGAAGGTGCTGC CTEGTGGTCTGGGGAAGCCCCTGGAACAGGGGGCGCAGGTCCCACACGGGTGCTCTGGCC TCCAGCTGCCAGGGGGGGCCGCGCTCAGGCCAGGGTCCCCTCCACCAGAACCGCCAGGGC .CCTGGGGAAAACCTGTCTGTGCTAACAGGGCCGCTCCCCGGGACTCCACGGAGAGGTGCU AGCGGAGGTGCCCGAAGGCCGGAAGAGCCCACCCTCCACTCGGGGACCTATTTCAGCAAGA AGACGGATGGGACTCCCGGGCATGGACAAAGGAACAGGATGAACCTTCTGGAACGCACAA GGCTTCCACGGCTGACCGGTCATAGGAAGGCGCGTCTCTAGGCCAATCCACCGTCCACCG TCCATTCCCCAGCCCTCGAGAGGGGGCAGGATGGACCGCTGCAGCGTGAGAGAGCTCTGG GGCGCTCCCACAGGGCAAAGTCCCAGGGCACTGACCTCAGAGCCCAACCAGGCCACCGGG GCTGGGCCCACCAGGGAGCCGGGGCCAGGGTCAGGGCCCAGAGTGCGGGAAACG GTGGCGTGTTGCTTGGGCCGGCGGGCGCGCAGACGGCCCCTCGCACCCCCGACAGCCCT GGAGCTGAGTGAAGCCCGCGGGTCACCTTGGCTGGGGTTGGGGTCTCCTGCGACCGGCAC CCCAGCTCAGGTCATCCTTGCTGTACCGCAGAGGGGCAGGGCTTCTGAGCAGGGACAGGG TGGGCCGCGCAGGAAGCCCCCTTCTCTCTGAGGCTGCCCCGGCCCTGGAGCCTCTCTGGG GCATGCCACCCCTCTCACAGACGCCTCCCAGGAGCCCCCACTTTCCTGCTGCGTGGTGAG TTGGAGCAGGTGCAGGGCATCACCACACAGCAGCAGAGGCTGTGGGGGCCCCTGAGAGGC GCTCCCAGGTACCCTCCTCAGGGGGCTGAGCCCGGGGTTGACCCGGGACCTCGCCTGCCC CAAAGCCGGCGCCTCCTCCCGCCCGCCCGACCAGGGCCAGAGAAGCAGGTGTGGGGCGG CACAAACCCAAGTCAGCTTCCAGATCCTGCTGGGGCCGCGTTGAAACTCGAAGCCCCCAG GCTGGGAGGTCTAGACACCCCTGCCCAGACCGACAGCCTGGGCCTGGCTCACAGCTGCCT GGGGGCCCAGGGGTGCACCTGCCCTGTGGGTGGGGGTCAGAGGGCAGGGAACCCTCGGGA AGGTCCCCAGGGTCAAGGTTGGGCCTAAGCTCCGGTGACCTCTGGGAAGTCTGGGGCTG GGTTTTGTTCCCAGAGGAGAGAGGGGCCAGTAGCCTCAGAGGGGCTGTGGCACGGTGGGAA GGCCCCAGGTGACCCCAGAGCGTGCGAAGCAAGCCCCCTTGACTGCAAAGC GCAAAGGGCAGAGGTGGGGTGGGAGCCTCGACCCCCGAGCCCAGGTACACAGGGGGAAG GGCGAGGGATCCGGCAGGGGCCACCCCGCCACGCAGGCCCACAAAGCCTTTGGGC CCGGAGCCCCAGATGGGCCCAGCCCAGCTCTGGGAACAGTCTTCCCAGAATTCCCCAGCT GGGATCTCCTAACTGGCAAGGCCTGTTGGGAGGGGCTGGTGAGAGGCCACTCTGGCGGGA AGACCCCAGCCACCTGGAGCCCCTAGCCACTGCCTGCTGCGGCTCCCTAGGGATCCAGG GCCATCAGAGAAGCTCCAGCGACACTGTTTATTTTCAAATGACACTTTTTAAGAAAAACA AGAGCCTGCACGGCCCTCCGGGTGGGGGGGGGGGGAGCACCCCTGGGACCGGGCCGC AGGCTGTCAGGGCACGGAACGTGTCTCTGGGCCCTGTCCTCAATTCCCGGTGCCCAGTGG CCCCAACTTCCCAGCAGACCCAGCAGGCCCCCAGCTTGTCTTGGCCTGGCCGCTGGTCCT GTCACCCCAGGCCTGGAGGTTCTGGAAGATTCTGCTCCTGCTCCCGTGTGCACATACCACT GCAGCCCGCCTGATCTTCCAGGTCCTCCTCCGAGCCCCCGCCTCCAGGAAGCCCTCCAGG AGAGCTCAGGAGGGTCGGCTCCCTGCGCGCAGCTGTCAGACCCCTGGGCCCACCCCGCCG GCTGCTAGGGTCCAGGTTCCCCACAAGCCCTCGGGCAGAGGCTGGGCCGCTGGGTCCCTC GGAGACAACTGGCTCCGAGGCCTTGCCCTAGACGGGTTTCCGGGAGCCCGTCCCCAGCGG

FIGURE 8. CONTD.

CACCCACTGAGTTTTGAACACTTGGCGCCACCCCCACACCCCAGGCGGTGGCCAGGAGGC CTCCTGGGCAGCAGACAGTCCGTGAGGTGGCCCTGGGGTGGCTCCTGACCTGGGCGCTGG CCCAGCCCTGGGCACAGCTTTCCAGATCTTGCCTGCCGCTTCCTCCAGGCTGCCTCGGCC CCTCCCGCCTGGGGGTGCCCAGCTTTTCCTGGAGGATGCCCACCCTTGCCCATGGTCAGG GAGGGGCTGAGAAACCCCACCTCGTGCCTGCCCGGCCTATGCCAGGGGAACCAGGTTC CCTCCCGCAGGAGGGGACCGAGTCCCTGACAGCCCACTGCAGAGGGGAGGAGGTGCCTGG CTCTGCCCCCAGCCCCACCAACCCCGTGGCTTCCTGTTTCGCAGCCCCACAAAGCACTAAA GGCCGCAGGTCCTGG/\MCATCAAAGACCCGGGAAGTCCAT'TGTATTGAATTGAGTGTAAA TGAGCCTGAGGCCTGTGGCTTGCGTTTCCCACAATTACCGCTGCCCGGGAAGGGCTCCGG NACCGACACAGCCCCCAGGGCCCCTTGCCCATGTGGGGAGCCCAGGCTGGCCTGAAGAAG CCCCATAAGGTGGACCCCACTTTGAGCCCCCACGAGAGTGGGCCAAGGACCAGGTCAGGG GCTGCCCAGGCTCTGGGCCTCCTGCCTGCCAGGTGGGCTCCCTCGGGGCCCAGCCTGG CCTGCAGGACCTTCCCACGCTGAGTTCCCCAGCCTGGTATGAGCGTAGTGGACGGCAGCC ATGCCCAGCACTCAGGGGCCTGAGGGGACAGAGCGGGAACTCCAGCCCCCGGGTCCTCGGC CCCTAGGATCCTTCTAGGTGGGGAAGCCCAAGGGAGCAGAGGGGGTGAACGCAGCTGTGTG GGGCCCAGGCTGCCGAGCAGACCCCTCCTGCTCCACTCCTCGGCCGAGTGGGCGCCGAG ATGCCGGGGCAGTGCCATTTCCCAGGCGCCACCGGAGGCTCCCAGAGGGAGTGAGGCACG GAGGCCCGGAGGGGCCTGGAGTCAATGACCCAGGGATTATCGTGCTGGGTCTTTGCAAA GTTGGCTGAGCAAACGCCGGAGCCAAGGGTCAGGGAGACGGGACTGGCGGGGCCCCGCGG CCCCCTTTCCCCTTTCTGGAAAAAGCCTGTTTCCCAGGTCAAAATCCAGCTCATGATCCG CCCCTTTGGGACTGATGTTCAGAGGCCCAGTCGTCCCAGCACCTCTGTCCACCGCCCCC CCCACGCTCCCGGGGCCGCCAACCCCTGTGGGGCTGCGAGGTGCGGGCACCTCTCCCTTCG AAGCAAAGCCCTGCCTGCGTGGGCAGCGTGATTTCCTGCTTCTCTGGGGCTGCACTTTG ACTGGGGTGGGGGGTGC

Contig 4 (1592 bp)

AGCCCCTCAGCCCCTCCGAGCAGCTGCTGGGCTCAGCGGGCTCGCCCCCGGATGTGCGGC CGGCCTCGGCCCCCTTCCCCGAGGGGCACCCCCACGGAGGGCCCAGACCGGAGGGACTC GGGGCCCAGAGGCCAGGGCAAGAGTGAAGGCAGCGCCGGTGGGAGCGGCGGTCAGCGGGG TCCAGGCTTCAGTTCCCAAGGAGCCCCATGCCCTGAGCCCGCACTGAGCCCTGTGCAGCC GGGGTGGCCTGACGGATGGTAACAGCTGCTCCCCCCCACCTCGCCGGCGTGGACAGGGCTC CCAGCCTCCCTCCTAATCCCCCGCATTTTCCGAATTCTCGGGCCACTGCTGCTTC CTCCTCAAATTCCTGGCCCCCTCGCCCCATCCCCGCCATGGGAAAGGGCCCGCGATGCCA GAGGTGCGGGGGTGCCAGGGAGAAGGGCCCAGATTAGGGGGCGTCATGGGAAAGCTGGGA GGGAACGCTACCCAGAGCCCCTCCTCCGCAGCCTGTGCTGCTCCCCTCTCCGCATTTCTG GCCTCTGAGTGCTCCCTGGAGGGAAGGGACCACTGTGTCCTGCCGGCCTCTGGCTCTGCC AGGAATGTCCATCTGTCCGGGCCGGGTTACCTGGCTCAGAGCGTGGGTACCAGCTCATCC AGCCCTGAGGCCTGCTCTCGGGAACAGTGGATGGGCCAGGCGCCCCCGTCACACCCCGGCA CCAGGAGCCCCAGGGACAGAGCCTCAGGGACAGACCCTCAGAGCCACAGCAGGAAGCCTG ACCCCGGGCCTCCTCTCGCACGATTCCCAGGCCAGCCTGGTCTCAGGCAGTCCAAGGTTG CACAATGGTCTCCATCCTCCAGAGTTGCAGAGCCAGCACTCTCCCACTGGACGGCGGCCC TTGACCCTGTCTCTTATACACATCTCAACCCTG

Contig 5 (831 bp)

44/48

FIGURE 8. CONTD. ;

Contig 6 (4634 bp)

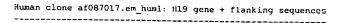
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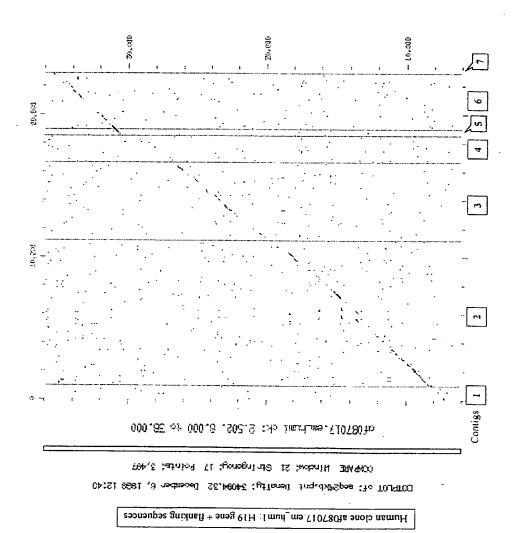
GAGAGGAGTTGGCGTGGACAGCAGGGGTCAGGCCCCTTTGCCCCGAGGGCAGGGCTGGTG CCACCTGGGTCAGGCGCAGGCCCTGGAAAAGCACCGGAAATGAGCACACCTGGGTCTCT AGAAGGTTCTTCCAGACCTCTGGGGGCTGAGTCATTTCAACACTCCTGGGCCGGGCAGGG CTTCTTCTTGGCCCCGAGGGACAAGGTCCCCTTCGTCCGGGGGGTACGGCCCCTGGACCC CCTCAGAGCCCCTTCTCCCTCCCTCCTCCTCCTCCTAGATGCCCCGGCCTCCAGG TGGGGCAGCCAGGCGGCAGAATGTGGTCCAGGCCTCTCGGCCCCACCCCACACCCCCCTGC TCTGCCCTGACAGCCTCCAAGACGCAGGCACGTCGCTGCGTTCTGCGTCCTCTCA TGGCACAAAACGGTGCCCCCCTAGCTTCCCCCAGAGAAGGGAGATCGTGCTCCCCGGACG GACCCTGCTCTGCCTGCCCGCCCGGCCTTCAGGGCCTCTCCCCAAGGGTGGCCGCGAGGACCCTCCGCCCACGGGGGCTCCATCCTCCCGAGCCCGACAGGCCTCCGCC TGGTGGTCCGACCTCTTCCCCAAGGCCCCGCCCATCCTCCTCGCGCTCCCCCAAACCCTG CCTCTTTCCCCAGCGCCCTTGTCCCCACGGAAGACCCTCCACCCGTGCCATTACACGGTC TGCCCGGGGGTCTCGCTGACCGCCTCCTACGGAAGCTGTGCCCGGGGGTGGGGGTGTCTC TGCCCGAACGGCTGGAGGACGAGCCACATCCCAGGGCAGCCGGAACCTGCGTCCTGGTCT GAGACGGAGAGGCTGGGTGCAGGTGGCTGAGGGGCCTGCACACAGCTTGGCCTGGGGTCC CCTAGGTGACAACACTGGCTGAACACTCATTGCTGCTCCCCTTCCAGGGTGACCCTGGGG TCCCCGTGTGGCCCTCAGGGCACACGGGGGCCCCACCAGGCCTCACAGAACCCCAGTGGG ACTGCACCCAGGGCCCACAGAAGTGCGGGGGCACTGGGGGTCCAGAAACAACCCCACAAC

CAGGCCAAGGTGGCCAAGGCCTTACTCGAGCGGGGCTGCCCAAGAGACTCTGGCC AGTCGTCCGGATCCAGCTTCCCGGGGCCGGGCCGCCGCTGGGCTCCAGGCGGTTCTGGG GGGCCCTCCCCGGGGGTTCGCCCTCCGCTCTCAGCAGCAGGAAGAGGAGCGCGGCCAGC GGATGGGGAGAAGAGGGCCCCTGGCCATCTTGCTCCCCCTGGGACTTGAGGAGGGTCTC GGGCCGGGCAGGCGGGACCGGGAGCCACAGAGACCCTGGAGGAGGCAGCATGGCGGGGAG GGAGGCGTGCCGCTGACCGCCTGGCCGGGAGGTTTGCTGCGTGTGGGGGTTTGCAGAAAGT CCTCGGGCACTGCTGACCCATCTCCCGTTTCCAGGGCACCAGAGCCACCTAATCTGCCGG CTCTGTGCCCAGGGACAGGCTTGCCTGATCTCTCAAGGCCGGGCGCTCCGCCTTCCCTGG GAGAGGGTTAAACATCCAGCCCCAGCCAGCATCTCGGGCAGGTTCCTGGCTCCCCCCGCT CGTGCCTCCTCTGAGACCCTGGTCGGCACACCTTTCCCTTGAGAGGAGGAGGAGGAGGAGGA AGCGGATGGAACCAGTGACCCTGCAGCCCCTGAGGGCACCTTCCCACGTGCCCCGGCCCG CGGGCTCCCTGGCTCCCCCCCGGGAAGACAGGGCCGCTCGGCTGGGGCTGCAGGGA GGGGCCCGAGACGCAGGAGAGCAGCCCGGAGGCAAACCCCGCGGGTCTTCCAGAAGGAGG CCTGGCAGGGGGGGGGGGGCCACCACTGCTGTCCCTCTCGTGCCACAGTGGAGGGTGT GGGTGGGCAGTGCCGGGTGGGAAGTGCAGAAAGACCCTGGACCGTGGGGCTGGGCCGCC CCGGATCACTTCCAGATTTGCTGTGGGACCAAGGGCCGGACCTCCGGGTGACTTCTTTTG TGTGCTGGCCACAGGGGGCCCCGGCGAGGUTCACACGGAAGGGGGCTTCGGACCTGGCCT AACAAGCCCACTCCCGAGGAAGATGCAAGGGGGAGGCCAGACGGAAGGGGCCGAAGGGGGCGA TCGGGGGACACCGCGGCAGGGCCGGGGCAGAGAGGGCAGAGGGCAGAGAAGGGACC CAGAGGGCAGAGAAGGGGCAGAGGGGCCACATGCTTGGAGGGCCAGGGAGGAGCGGGA ACGGCGTCCGGCGTCCAGCGCCGAATCAGGCCCGTCAGGCGGAGGGTGCGTGGACCTGCC TGGCCTTCACGAGCACAGTCAGCAGGCTGTCTCTTATACACATCTCAACCATCAT

Contig 7 (482 bp)

FIGURE 9





SUBSTITUTE SHEET (RULE 26)

FIGURE 10

IDENTIFIED POLYMORPHISMS:

POLYMORPHISMS TYROSINE HYDROXYLASE GENE - CONTIG C3 (figure 6)					
1	GGATCCAGCC(A:T)GCAGCC	1081	bp		
2	ACAACCCCC(-:C)TCCCACAG	1149	pp		
3	TGCGGAGGGG(A:G)GACCTG	1186	bp		
4	AGGT (CAAGGCCAGGT:-) CGAGG	1210	рb		
<u>POLYMORPHIS</u>	MS INSULIN-IGF2 - CONTIG C4 (figure	6)			
5	CCC (C:A) CCCC (A:C) CGCCGC	438	рb		
6	CCC (C:A) CCCC (A:C) CGCCGC	443	bp		
7	CGCCGCAGCA (G:A) GCCG	455	pd		
8	GCTTATGG (G: A) GCCGGG	503	pp		
9	CACGGC (T:C) TC (G:A) GAGCA	525	bp		
10	CACGGC (T:C) TC (G:A) GAGCA	528	рb		
11	GTCTGC (A:G) GGCAGGTG	571	рb		
12	CAAGCCCGG (G:T) CGGTT	636	рb		
13	ACCTC (A:G) AGGCCCCCA	710	qα		
14	GC (C:T) GGGCCCAGCCGC	867	рp		
15	ACCAGCTG (C:T) GTTCCC	903	bp		
16	GGC (C:G) CTCTGGGCGCC	1148	bp		
17	GGGGG (C:T) GTCCCGGGA	1305	bp		

SUBSTITUTE SHEET (RULE 26)

MICROSATELLITES

29	PIGQTL1:	(AT) 11		112	to	133	рþ	Contig	57	
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2248 bp

2257 bp

2413 bp

CGCCCCCCC(-:C) (G:C)GG

GCAGCCGGCTC(C:T)TGG

GTTGTTG (C:T) TCTGGGA

30 PIGQTL2: (GT) 8 GCACGCGTGTGCGTGTGTAC (GT) 17 1074 to 1144 bp Contig

95

26

27

28

31 PIGQTL3: (CA) 19 223 to 260 bp Contig 105

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